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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Ayako TODA, et al.
SERIAL NO.: NEW U.S. PCT APPLICATION
FILED: HEREWITH
INTERNATIONAL APPLICATION NO.: PCT/JP01/01204
INTERNATIONAL FILING DATE: February 20, 2001
FOR: CYCLIC HEXAPEPTIDE DERIVATIVES

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119 AND THE INTERNATIONAL CONVENTION

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
Australia	PQ5752	21 February 2000
Australia	PQ9552	21 August 2000
Australia	PR2344	28 December 2000

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/JP01/01204. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

Respectfully submitted,
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I, JONNE YABSLEY, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 5752 for a patent by FUJISAWA PHARMACEUTICAL CO., LTD filed on 21 February 2000.

WITNESS my hand this
Twentieth day of February 2001

J R Yabsley

JONNE YABSLEY
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EXAMINATION SUPPORT & SALES

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Regulation 3.2

Fujisawa Pharmaceutical Co., Ltd.

AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"New Compound"

The invention is described in the following statement:

DESCRIPTION

NEW COMPOUND

TECHNICAL FIELD

The present invention relates to new polypeptide compounds and salts thereof which are useful as a medicament.

BACKGROUND ART

In U.S. Pat. No. 5,376,634, 5,569,646, WO 96/11210 and WO 99/40108, there are disclosed the polypeptide compound and a pharmaceutically acceptable salt thereof, which have antimicrobial activities (especially antifungal activity).

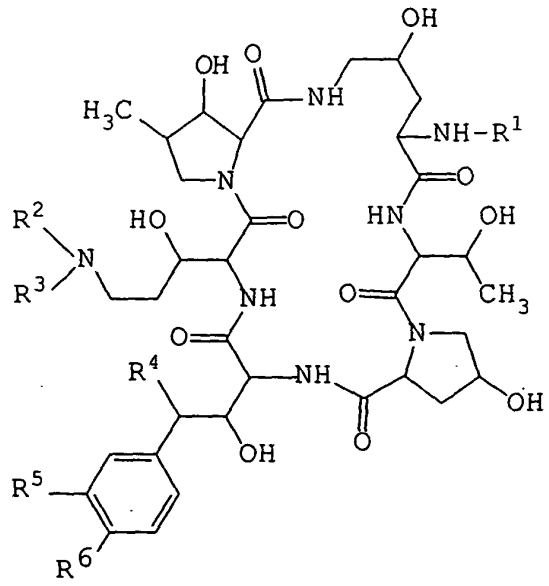
DISCLOSURE OF INVENTION

The present invention relates to new polypeptide compound and a salt thereof.

More particularly, it relates to new polypeptide compound and a salt thereof, which have antimicrobial activities [especially, antifungal activities, in which the fungi may include Aspergillus, Cryptococcus, Candida, Mucor, Actinomyces, Histoplasma, Dermatophyte, Malassezia, Fusarium and the like.], inhibitory activity on β-1,3-glucan synthase, and further which are expected to be useful for the prophylactic and/or therapeutic treatment of Pneumocystis carinii infection (e.g. Pneumocystis carinii pneumonia) in a human being or an animal, to a process for preparation thereof, to a pharmaceutical composition comprising the same, and to a methods for the prophylactic and/or therapeutic treatment of infectious disease including Pneumocystis carinii infection (e.g. Pneumocystis carinii pneumonia) in a human being or an animal.

The object polypeptide compounds of the present invention are new and can be represented by the following

general formula (I) :



(I)

wherein

R^1 is hydrogen or acyl group,

R^2 and R^3 are independently hydrogen, lower alkyl which may have one or more suitable substituent(s) or acyl group,

R^4 is hydrogen or hydroxy, and

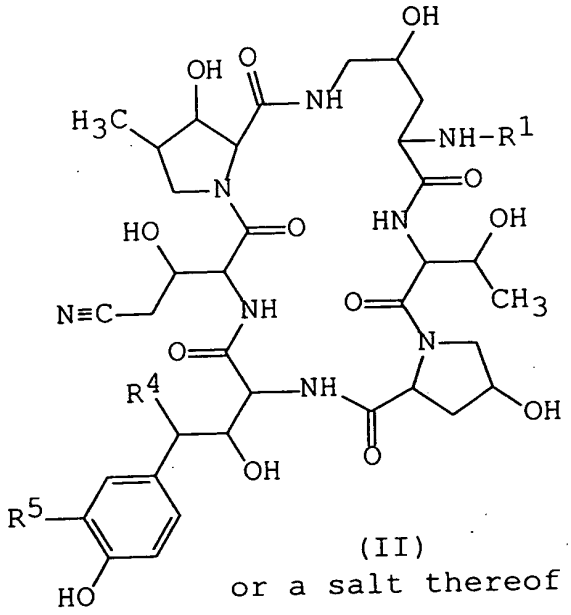
R^5 is hydrogen, hydroxy or hydroxysulfonyloxy,

R^6 is hydroxy or acyloxy,

or a salt thereof.

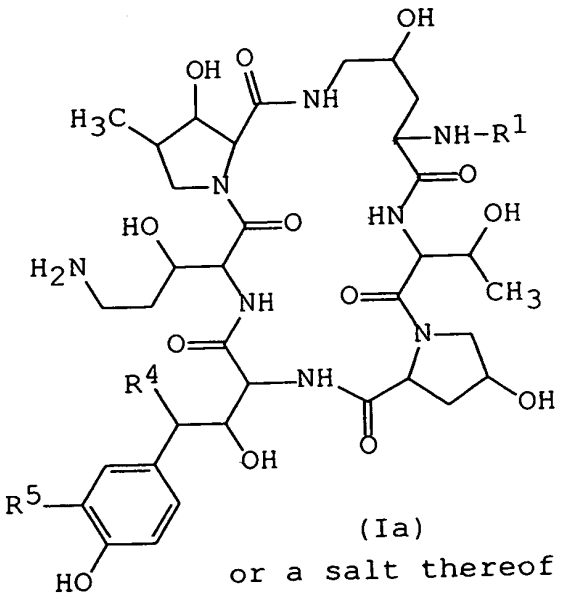
The new polypeptide compound (I) or a salt thereof can be prepared by the process as illustrated in the following reaction schemes.

Process 1



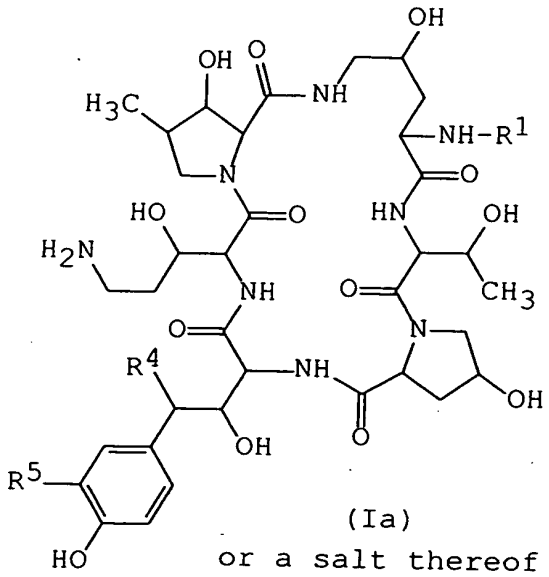
(II)
or a salt thereof

reduction



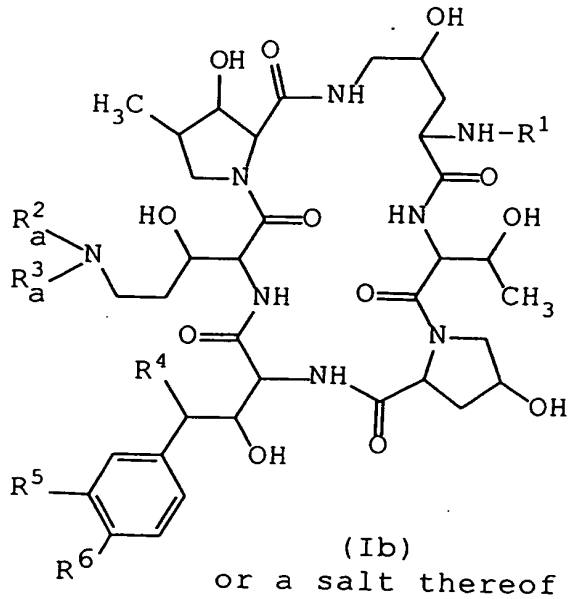
(Ia)
or a salt thereof

Process 2



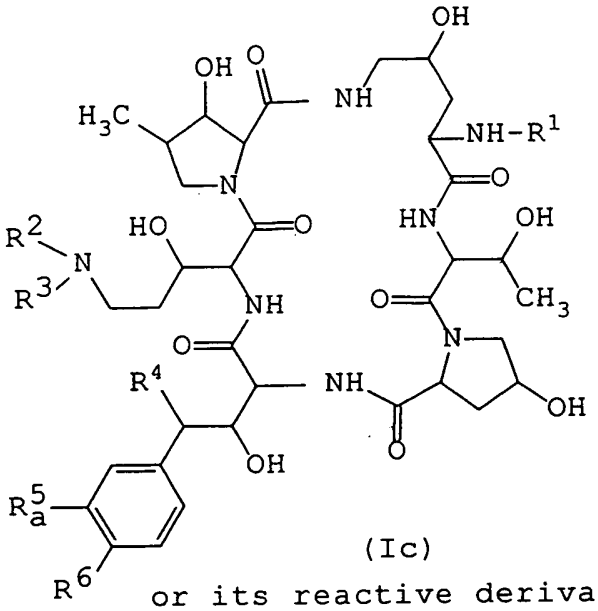
(Ia)
or a salt thereof

protective reaction



(Ib)
or a salt thereof

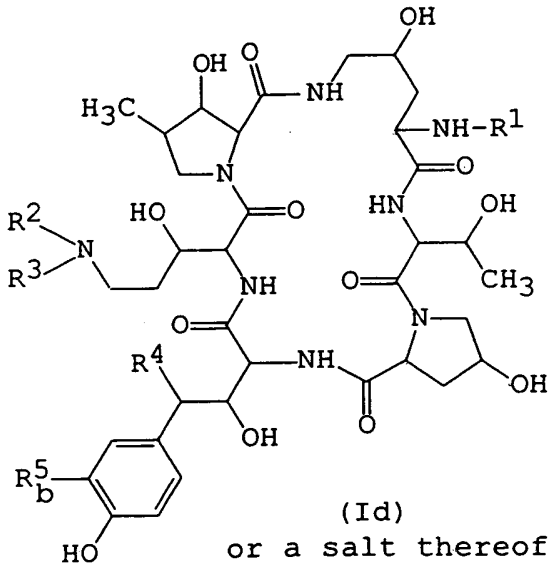
Process 3



hydrolysis

(Ic)

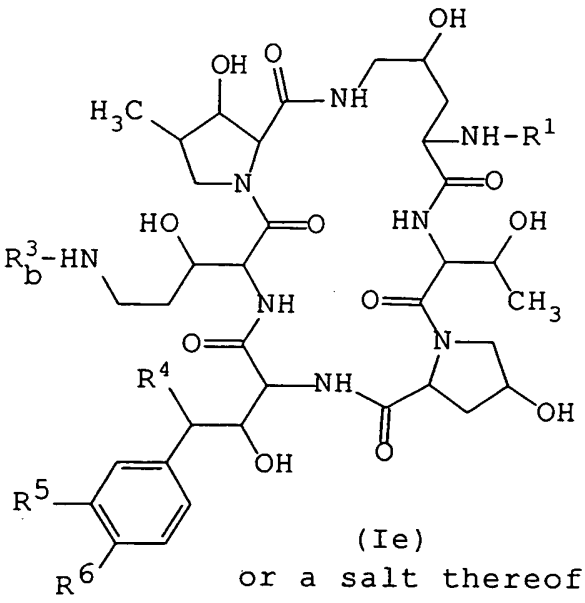
or its reactive derivative at the sulfonic acid group or a salt thereof



(Id)

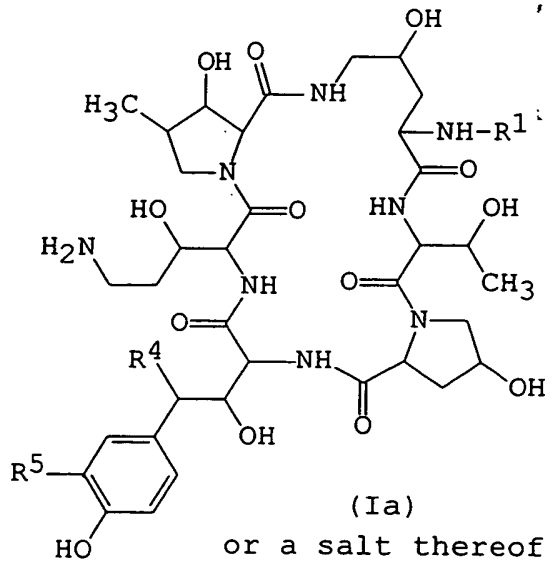
or a salt thereof

Process 4



(Ie)
or a salt thereof

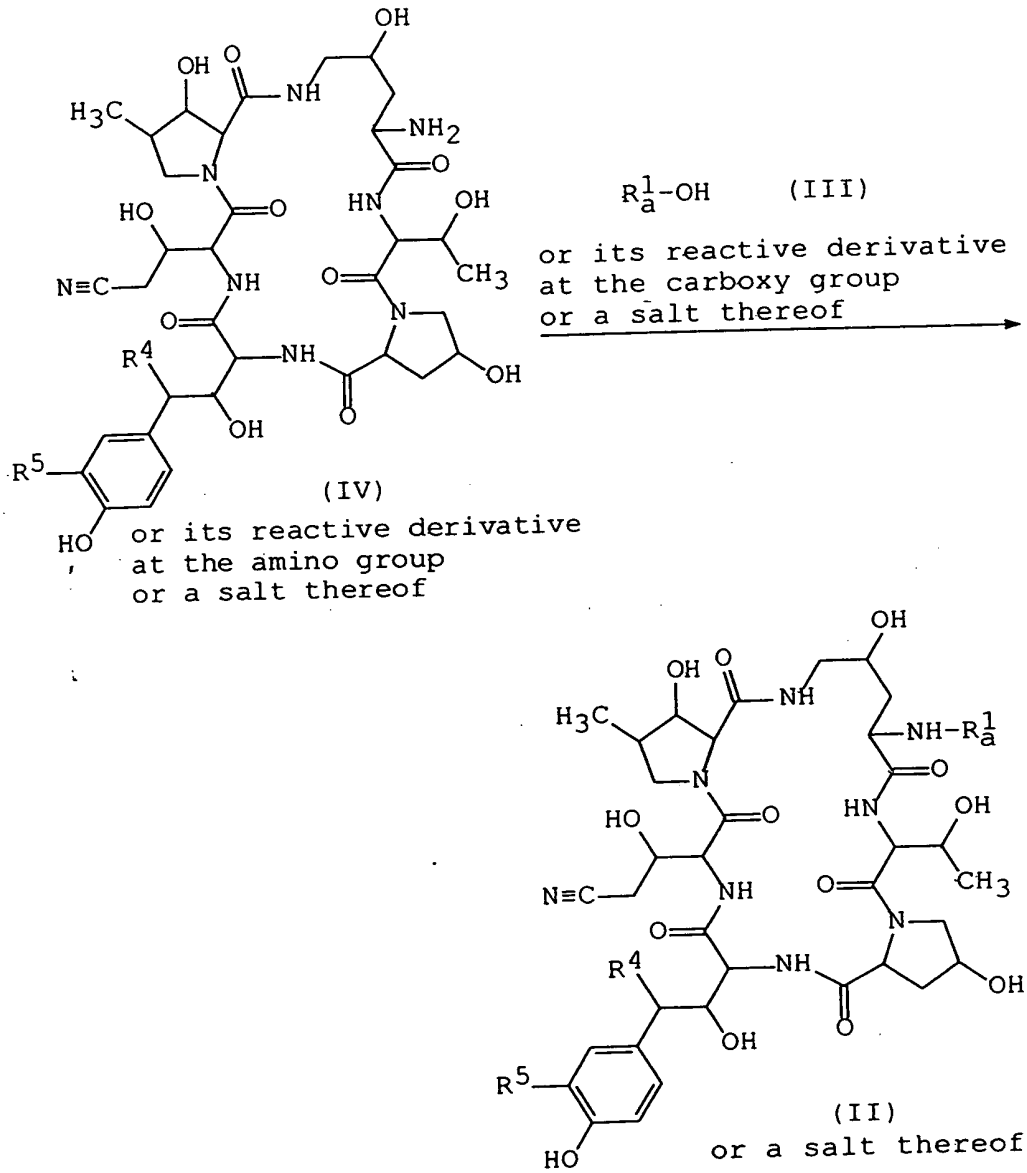
elimination reaction



(Ia)
or a salt thereof

The Starting compound (II) or a salt thereof can be prepared by the process as illustrated in the following reaction scheme.

Process A



wherein R^1, R^2, R^3, R^4, R^5 and R^6 are defined above,

R^1_a is acyl group,

R_a^2 is hydrogen, lower alkyl which may have one or more suitable substituent(s) or acyl group,
R_a^3 is lower alkyl which may have one or more suitable substituent(s) or acyl group,
R_b^3 is amino protective group,
R_a^5 is hydroxysulfonyloxy, and
R_b^5 is hydroxy.

Suitable salt of the new polypeptide compound (I) is a pharmaceutically acceptable and conventional non-toxic salt, and may include a salt with a base or an acid addition salt such as a salt with an inorganic base, for example, an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, magnesium salt, etc.), an ammonium salt;
a salt with an organic base, for example, an organic amine salt (e.g., triethylamine salt, diisopropylethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, , N,N'-dibenzylethylenediamine salt, etc.);
an inorganic acid addition salt (e.g., hydrochloride hydrobromide, sulfate, phosphate, etc.);
an organic carboxylic sulfonic acid addition salt (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.);
a salt with a basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid, etc.).

Suitable examples and illustration of the various definitions in the above and subsequent descriptions of the present specification, which the present invention intends to include within the scope thereof, are explained in detail as follows :

The term "lower" is used to intend a group having 1 to 6

carbon atom(s), unless otherwise provided.

Suitable example of "one or more" may be the number of 1 to 6, in which the preferred one may be the number of 1 to 3.

Suitable example of "lower alkoxy" may include straight or branched one such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tert-butoxy, pentyloxy, tert-pentyloxy, neo-pentyloxy, hexyloxy, isohexyloxy and the like.

Suitable example of "higher alkoxy" may include straight or branched one such as heptyloxy, octyloxy, 3,5-dimethyloctyloxy, 3,7-dimethyloctyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, hexadecyloxy, heptadecyloxy, octadecyloxy, nonadecyloxy, icosyloxy, and the like.

Suitable example of "lower alkyl" may include straight or branched one having 1 to 6 carbon atom(s), such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, tert-pentyl, neo-pentyl, hexyl, isohexyl and the like.

Suitable example of "aryl" and "ar" moiety may include phenyl which may have lower alkyl (e.g., phenyl, mesityl, xylyl, tolyl, etc.), naphthyl, anthryl, indanyl, fluorenyl and the like.

Suitable example of "aroyl" may include benzoyl, toluoyl, naphthoyl, anthrylcarbonyl, and the like.

Suitable example of "heterocyclic group" may include

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g., 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.), tetrazolyl (e.g. 1H-tetrazolyl, 2H-tetrazolyl, etc.), etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolidinyl, imidazolidinyl,

piperidyl, piperazinyl, azetidinyl, etc.;

unsaturated condensed heterocyclic group containing 1 to 4 nitrogen atom(s), for example, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl (e.g., 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.), etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, morpholinyl, sydnonyl, morpholino, etc.;

unsaturated condensed heterocyclic group containing 1 or 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, benzoxazolyl, benzoxadiazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, isothiazolyl, thiadiazolyl (e.g., 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.), dihydrothiazinyl, etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example thiazolidinyl, thiomorpholinyl, thiomorpholino, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 sulfur atom(s), for example, thienyl, dihydrodithiinyl, dihydrodithionyl, etc.;

unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, benzothiazolyl, benzothiadiazolyl, imidazothiadiazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-

membered) heteromonocyclic group containing an oxygen atom, for example, furyl etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 or 2 oxygen atom(s), for example, tetrahydrofuran, tetrahydropyran, dioxacyclopentane, dioxacyclohexane, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing an oxygen atom and 1 or 2 sulfur atom(s), for example, dihydrooxathiinyl, etc.;

unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s), for example benzothienyl, benzodithiinyl, etc.;

unsaturated condensed heterocyclic group containing an oxygen atom and 1 or 2 sulfur atom(s), for example, benzoxathiinyl, etc.; and the like and this "heterocyclic group" may have one or more lower alkyl.

Suitable example of "cyclo(lower)alkyl" may include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like.

Suitable example of "cyclo(lower)alkyloxy" may include cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like.

Suitable example of "acyl group" may include aliphatic acyl, aromatic acyl, arylaliphatic acyl and heterocyclic-aliphatic acyl derived from carboxylic acid, carbonic acid, carbamic acid, sulfonic acid, and the like.

Suitable example of said "acyl group" may be illustrated as follows.

Aliphatic acyl such as lower or higher alkanoyl (e.g., formyl, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl,

tridecanoyl, tetradecanoyl, pentadecanoyl, palmitoyl, heptadecanoyl, octadecanoyl, nonadecanoyl, icosanoyl, etc.);
lower or higher alkoxycarbonyl (e.g., methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, t-pentyloxycarbonyl, heptyloxycarbonyl, etc.);
lower alkenyloxycarbonyl (e.g., vinyloxycarbonyl, propenyloxycarbonyl, allyloxycarbonyl, butenyloxycarbonyl, butadienyloxycarbonyl, pentenyloxycarbonyl, hexenyloxycarbonyl, etc.);
lower or higher alkylsulfonyl (e.g., methylsulfonyl, ethylsulfonyl, etc.);
lower or higher alkoxysulfonyl (e.g., methoxysulfonyl, ethoxysulfonyl, etc.); or the like;

Aromatic acyl such as

aroyl (e.g., benzoyl, toluoyl, naphthoyl, etc.);
ar(lower)alkanoyl [e.g., phenyl(C_1-C_6)alkanoyl (e.g., phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutanoyl, phenylpentanoyl, phenylhexanoyl, etc.), naphthyl(C_1-C_6)alkanoyl (e.g., naphthylacetyl, naphthylpropanoyl, naphthylbutanoyl, etc.), etc.];
ar(lower)alkenoyl [e.g., phenyl(C_3-C_6)alkenoyl (e.g., phenylpropenoyl, phenylbutenoyl, phenylmethacryloyl, phenylpentanoyl, phenylhexenoyl, etc.), naphthyl(C_3-C_6)alkenoyl (e.g., naphthylpropenoyl, naphthylbutenoyl, etc.), etc.];
ar(lower)alkoxycarbonyl [e.g., phenyl(C_1-C_6)alkoxycarbonyl (e.g., benzyloxycarbonyl, etc.), fluorenyl(C_1-C_6)alkoxy-carbonyl (e.g., fluorenylmethyloxycarbonyl, etc.), etc.];
aryloxycarbonyl (e.g., phenoxycarbonyl, naphthyloxycarbonyl, etc.);
aryloxy(lower)alkanoyl (e.g., phenoxyacetyl, phenoxypropionyl, etc.);
arylcarbamoyl (e.g., phenylcarbamoyl, etc.);
arylthiocarbamoyl (e.g., phenylthiocarbamoyl, etc.);
arylglyoxyloyl (e.g., phenylglyoxyloyl, naphthylglyoxyloyl,

etc.);
arylsulfonyl which may have 1 to 4 lower alkyl (e.g., phenylsulfonyl, p-tolylsulfonyl, etc.); or the like;

Heterocyclic acyl such as
heterocycliccarbonyl;
heterocyclic(lower)alkanoyl (e.g., heterocyclicacetyl, heterocyclicpropanoyl, heterocyclicbutanoyl, heterocyclicpentanoyl, heterocyclichexanoyl, etc.);
heterocyclic(lower)alkenoyl (e.g., heterocyclicpropenoyl, heterocyclicbutenoyl, heterocyclicpentenoyl, heterocyclichexenoyl, etc.);
heterocyclicglyoxyloyl; or the like;
in which suitable "heterocyclic" moiety in the terms "heterocycliccarbonyl", "heterocyclic(lower)alkanoyl", "heterocyclic(lower)alkenoyl" and "heterocyclicglyoxyloyl" can be referred to aforementioned "heterocyclic" moiety, and these "acyl group" may have one or more suitable substituent(s) selected from the group consisting of lower alkyl, oxo, and amino.

Suitable example of "acyl group" of R^1 can be referred to aforementioned "acyl group", in which the preferred one may be higher alkanoyl, lower alkoxycarbonyl, aroyl which may have one or more suitable substituent(s) and the like.

Suitable example of "suitable substituent(s)" in the term of "aroyl substituted with one or more suitable substituent(s)" may be heterocyclic group substituted with aryl having lower alkoxy, heterocyclic group substituted with aryl having lower alkoxy(lower)alkoxy, heterocyclic group substituted with aryl having lower alkoxy(higher)alkoxy, heterocyclic group substituted with aryl having cyclo(lower)alkyloxy, heterocyclic group substituted with aryl having heterocyclic group, heterocyclic group substituted with cyclo(lower)alkyl having cyclo(lower)alkyl,

heterocyclic group substituted with aryl having aryl substituted with lower alkoxy(lower)alkoxy, heterocyclic group substituted with aryl having heterocyclic group substituted with cyclo(lower)alkyl having lower alkyl and heterocyclic group substituted with aryl having heterocyclic group substituted with lower alkoxy(lower)alkoxy, in which the preferred one may be unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having (C_4-C_6)alkoxy, unsaturated 3 to 8-membered heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having (C_1-C_4)alkoxy(C_4-C_6)alkoxy, unsaturated 3 to 8-membered heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having (C_1-C_4)alkoxy(C_7-C_{14})alkoxy, saturated 3 to 8-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s) substituted with phenyl having (C_1-C_4)alkoxy(C_7-C_{14})-alkoxy, unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having cyclo(C_4-C_6)alkyloxy, unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having saturated 3 to 8-membered heteromonocyclic group containing 1 or 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), saturated 3 to 8-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s) substituted with cyclo(C_4-C_6)alkyl having cyclo(C_4-C_6)alkyl, unsaturated 3 to 8-membered heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having phenyl substituted with (C_1-C_4)alkoxy(C_1-C_4)alkoxy, unsaturated 3 to 8-membered heteromonocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having saturated 3 to 8-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s) substituted with cyclo(C_4-C_6)alkyl having (C_1-C_4)alkyl, unsaturated condensed

heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having saturated 3 to 8-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s) having cyclo(C_4-C_6)alkyl and unsaturated condensed heterocyclic group containing 1 or 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) substituted with phenyl having saturated 3 to 8-membered heteromoncyclic group 1 to 4 nitrogen atom(s) substitued with (C_1-C_4)alkoxy(C_4-C_6)alkoxy, and the most preferred one may be imidazothiadiazolyl substituted with phenyl having pentyloxy, thiadiazolyl substituted with phenyl having methoxyhexyloxy, thiadiazolyl substituted with phenyl having methoxyoctyloxy, thiadiazolyl substituted with phenyl having methoxyheptyloxy, imidazothiadiazolyl substituted with phenyl having cyclohexyloxy, imidazothiadiazolyl substituted with phenyl having dimethylmorpholino, piperazinyl substituted with phenyl having methoxyheptyloxy, piperazinyl substituted with phenyl having methoxyoctyloxy, piperazinyl substituted with cyclohexyl having cyclohexyl, thiadiazolyl substituted with phenyl having phenyl substituted with methoxyethoxy, thiadiazolyl substituted with phenyl having phenyl substituted with methoxybutoxy, thiadiazolyl substituted with phenyl having phenyl substituted with ethoxypropoxy, imidazothiadiazolyl substituted with phenyl having piperazinyl substituted with cyclohexyl, imidazothiadiazolyl substituted with phenyl having piperazinyl substituted with cyclohexyl, thiadiazolyl substituted with phenyl having piperazinyl substiuted with methylcyclohexyl and imidazothiadiazolyl substituted with phenyl having piperidyl substituted with methoxypentyloxy.

The more suitable example of "acyl group" may be palmitoyl, tert-butoxycarbonyl, benzoyl which has imidazolthiadiazolyl substituted with phenyl having pentyloxy, benzoyl which has thiadiazolyl substituted with phenyl having methoxyhexyloxy, benzoyl which has thiadiazolyl substituted with phenyl having methoxyoctyloxy, benzoyl which

has thiadiazolyl substituted with phenyl having methoxyheptyloxy, benzoyl which has imidazothiadiazolyl substituted with phenyl having cyclohexyloxy, benzoyl which has imidazothiadiazolyl substituted with phenyl having dimethylmorpholino, benzoyl which has piperazinyl substituted with phenyl having methoxyheptyloxy, benzoyl which has piperazinyl substituted with phenyl having methoxyoctyloxy, benzoyl which has piperazinyl substituted with cyclohexyl having cyclohexyl, benzoyl which has thiadiazolyl substituted with phenyl having phenyl substituted with methoxyethoxy, benzoyl which has thiadiazolyl substituted with phenyl having phenyl substituted with methoxybutoxy, benzoyl which has thiadiazolyl substituted with phenyl having phenyl substituted with ethoxypropoxy, benzoyl which has imidazothiadiazolyl substituted with phenyl having piperazinyl substituted with cyclohexyl, benzoyl which has imidazothiadiazolyl substituted with phenyl having piperazinyl substituted with cyclohexyl, benzolyl which has thiadiazolyl substituted with phenyl having piperazinyl substituted with methylcyclohexyl and benzoyl which has imidazothiadiazolyl substituted with phenyl having piperidyl substituted with methoxypentyloxy.

Suitable example of "lower alkyl" in the term of "lower alkyl which may have one or more suitable substituent(s)" can be referred to aforementioned "lower alkyl", in which the preferred one may be methyl, ethyl, propyl, isopropyl, buthyl, pentyl and hexyl.

Suitable example of "suitable substituent(s)" in the term of "lower alkyl which may have one or more suitable substituent(s)" may be imino, amino, carbamoyl, lower alkoxy, hydroxy, and the like.

Suitable example of "lower alkyl which may have one or more suitable substituent(s)" may be iminomethyl, 1-iminoethyl, amidino, 1-imino-2-carbamoylethyl, 1-imino-3-

methoxypropyl, carboxymethyl, 3-aminopropyl, 1-methylpyrazol-4-ylmethyl, dihydoroxyisopropyl, 2,3-dihydroxypropyl, 2,3,4-trihydroxybutyl, 2,3,4,5-tetrahydroxypentyl, 2,3,4,5,6-pentahydroxyhexyl.

Suitable example of "acyl group" of R^2 and R^3 can be referred to aforementioned "acyl group", in which the preferred one may be "amino protective group" mentioned below, and the most preferred one may be acetyl, 2-acetyloxypropionyl, methylsulfonyl, 2,5-diaminopentanoyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, allyloxycarbonyl and tert-butoxycarbonyl.

Suitable example of "amino protective group" may be a conventional protective group such as ar(lower)alkoxycarbonyl, lower alkenyloxycarbonyl and lower alkoxycarbonyl, in which the preferred one may be phenyl(C_1-C_4)alkoxycarbonyl, fluorenyl(C_1-C_4)alkoxycarbonyl, (C_2-C_4)alkenyloxycarbonyl and (C_1-C_4)alkoxycarbonyl, and the most preferred one may be benzyloxycarbonyl, fluorenylmethoxycarbonyl, allyloxycarbonyl and tert-butoxycarbonyl.

Suitable example of "acyl" moiety of "acyloxy" can be referred to aforementioned "acyl", in which the preferred one may be lower alkenyloxycarbonyl, and the most preferred one may be allyloxycarbonyl.

Suitable example of "acyoxy" may be lower alkenyloxycarbonyloxy, and the most preferred one may be allyloxycarbonyloxy.

Process 1

The object compound (Ia) or a salt thereof can be prepared by reducing a compound (II) or a salt thereof.

Suitable salts of the compounds (Ia) and (II) may be the

same as those exemplified for the compound (I).

The reaction can be carried out in a conventional manner namely, chemical reduction or catalytic reduction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, hydride transfer reagent such as aluminum hydride compound (e.g. lithium aluminum hydride, lithium hydridotri-t-butoxyaluminate, etc.), borohydride compound (e.g. sodium borohydride, sodium cyanoborohydride, etc.) or the like etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalyst [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalyst [e.g., spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.], nickel catalyst [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalyst [e.g. reduced cobalt, Raney cobalt, etc.], iron catalyst [e.g. reduced iron, Raney iron, etc.], copper catalyst [e.g. reduced copper, Raney copper, Ullman copper, etc.] or the like.

The reaction of this process is usually carried out in a solvent such as water, alcohol [e.g. methanol, ethanol, propanol, etc.], acetic acid, diethyl ether, dioxane, tetrahydrofuran, methylene chloride, etc. or a mixture thereof.

The reaction is preferably carried out under somewhat milder conditions such as under cooling to warming.

It is included within the scope of the present invention that "hydroxy" in R^4 may be reduced to "hydrogen" during the

reaction.

Process 2

The object compound (Ib) or a salt thereof can be prepared by subjecting the compound (Ia) or a salt thereof to protective reaction of amino.

This protective reaction may include acylation or alkylation reaction of amino, and can be carried out according to a conventional manner such as the one described in Examples or the similar manners thereto.

Process 3

The object compound (Id) or a salt thereof can be prepared by subjecting the compound (Ic) or its reactive derivative at the sulfonic acid group or a salt thereof to hydrolysis reaction of the sulfonic acid group.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g., sodium potassium, etc.], an alkaline earth metal [e.g., magnesium, calcium, etc.], the hydroxide or carbonate or hydrogencarbonate thereof, trialkylamine [e.g. trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, or the like.

Suitable acid may include an organic acid [e.g., formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], and an inorganic acid [e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g., trichloroacetic acid, trifluoroacetic acid, etc.], or the like preferably carried out in the presence of cation trapping agent [e.g., anisole, phenol, etc.].

The reaction is usually carried out in a conventional

solvent such as water, alcohol [e.g. methanol, ethanol, isopropyl alcohol, etc.], tetrahydrofuran, dioxane, toluene, methylene chloride, ethylene dichloride, chloroform, N,N-dimethylformamide, N,N-dimethylacetamide or any other organic solvent which do not adversely affect the reaction, or the mixture thereof.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

Process 4

The object compound (Ia) or a salt thereof can be prepared by subjecting a compound (Ie) or a salt thereof to elimination reaction of amino protective group.

This reaction is carried out in accordance with a conventional method such as hydrolysis, reduction or the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid. Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g. sodium, potassium, etc.], an alkaline earth metal [e.g. magnesium, calcium, etc.], the hydroxide or carbonate or bicarbonate thereof, trialkylamine [e.g. trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, or the like.

Suitable acid may include an organic acid [e.g. formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.] and an inorganic acid [e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.]. The elimination using Lewis acid such as trihaloacetic acid [e.g. trichloroacetic acid, trifluoroacetic acid, etc.] or the like is preferably carried out in the presence of cation trapping agents [e.g. anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as

water, an alcohol [e.g. methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as the solvent. The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

The reduction method applicable for the elimination reaction may include chemical reduction and catalytic reduction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g. spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium, sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g. reduced cobalt, Raney cobalt, etc], iron catalysts [e.g. reduced iron, Raney iron, etc], copper catalysts [e.g. reduced copper, Raney copper, Ullman copper, etc.] and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide, or a mixture thereof. Additionally, in case that the above-mentioned acids to be used in chemical reduction are in liquid, they can also be used as a solvent. Further, a suitable solvent to be used in catalytic reduction may be the

above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc., or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling to warming.

Process A

The object polypeptide compound (II) or a salt thereof can be prepared by reacting the compound (IV) or its reactive derivative at the amino group or a salt thereof with the compound (III) or its reactive derivative at the carboxy group or a salt thereof.

Suitable reactive derivative at the carboxy group of the compound (III) may include an acid halide, an acid anhydride, an activated amide, an activated ester, and the like. Suitable examples of the reactive derivatives may be an acid chloride; an acid azide; a mixed acid anhydride with an acid such as substituted phosphoric acid [e.g., dialkylphosphoric acid, phenylphosphoric acid, diphenylphosphoric acid, dibenzylphosphoric acid, halogenated phosphoric acid, etc.], dialkylphosphorous acid, sulfurous acid, thiosulfuric acid, sulfuric acid, sulfonic acid [e.g., methanesulfonic acid, etc.], aliphatic carboxylic acid [e.g., acetic acid, propionic acid, butyric acid, isobutyric acid, pivaric acid, pentanoic acid, isopentanoic acid, 2-ethylbutyric acid trichloroacetic acid, etc.]; or aromatic carboxylic acid [e.g., benzoic acid, etc.]; a symmetrical acid, anhydride; an activated amide with imidazole, 4-substituted imidazole, dimethylpyrazole, triazole, tetrazole or 1-hydroxy-1H-benzotriazole; or an activated ester [e.g., cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl [$(CH_3)_2\overset{+}{N}=CH-$] ester, vinyl ester, propargyl ester, p-nitrophenyl ester, 2,4-dinitrophenyl ester, trichlorophenyl ester, pentachloropentyl ester, mesylphenyl ester, phenylazophenyl

ester, phenyl thioester, p-nitrophenyl thioester, p-cresyl thioester, carboxymethyl thioester, pyranyl ester, pyridyl ester, piperidyl ester, 8-quinolyl thioester, etc.], or an ester with a N-hydroxy compound [e.g. N,N-dimethylhydroxylamine, 1-hydroxy-2-(1H)-pyridone, N-hydroxysuccinimide, N-hydroxyphthalimide, 1-hydroxy-1H-benzotriazole, etc.], and the like. These reactive derivatives can optionally be selected from them according to the mind of the compound (III) to be used.

Suitable salts of the compound (III) and its reactive derivative can be referred to the ones as exemplified for the object polypeptide compound (I).

The reaction is usually carried out in a conventional solvent such as water, alcohol [e.g., methanol, ethanol, etc.], acetone, dioxane, acetonitrile, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely influence the reaction. These conventional solvent may also be used in a mixture with water.

In this reaction, when the compound (III) is used in a free acid form or its salt form, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide; N-cyclohexyl-N'-morpholinoethylcarbodiimide; N-cyclohexyl-N'-(4-diethylaminocyclohexyl)carbodiimide; N,N'-diethylcarbodiimide; N,N'-diisopropylcarbodiimide; N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; N,N-carbonylbis-(2-methylimidazole); pentamethyleneketene-N-cyclohexylimine; diphenylketene-N-cyclohexylimine, ethoxyacetylene; 1-alkoxy-2-chloroethylene; trialkyl phosphite; ethyl polyphosphate; isopropyl polyphosphate; phosphorus oxychloride (phosphoryl chloride); phosphorus trichloride; thionyl chloride; oxalyl chloride;

lower alkyl haloformate [e.g., ethyl chloroformate, isopropyl chloroformate, etc.]; triphenylphosphine; 2-ethyl-7-hydroxybenzisoxazolium salt; 2-ethyl-5-(m-sulfophenyl)isoxazolium hydroxide intramolecular salt; 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole; so-called Vilsmeier reagent prepared by the reaction of N,N-dimethylformamide with thionyl chloride, phosgene, trichloromethyl chloroformate, phosphorous oxychloride, methanesulfonyl chloride, etc.; or the like.

The reaction may also be carried out in the presence of an inorganic or organic base such as an alkali metal carbonate, alkali metal bicarbonate, tri(lower)alkylamine (eg., triethylamine, diisopropylethylamine, etc.), pyridine, di(lower)alkylaminopyridine (e.g., 4-dimethylaminopyridine, etc.), N-(lower)alkylmorpholine, N,N-di(lower)alkylbenzylamine, or the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

The compounds obtained by the above Processes 1 to 4 and Process A can be isolated and purified by a conventional method such as pulverization, recrystallization, column-chromatography, high-performance liquid chromatography (HPLC), reprecipitation, desalting resin column chromatography, or the like.

The compounds obtained by the above Processes 1 to 4 and Process A may be obtained as its solvate, such as hydrate, and its solvate, such as hydrate is included within the scope of present invention.

It is to be noted that each of the object compound (I) may include one or more stereoisomer such as optical isomer(s) and geometrical isomer(s) due to asymmetric carbon atom(s) and double bond(s) and all such isomers and the mixture thereof are included within the scope of the present invention.

The object compound (I) or a salt thereof includes solvated compound [e.g., enclosure compound (e.g., hydrate, etc.)].

The object compound (I) or a salt thereof includes both its crystal form and non-crystal form.

It should be understood that the compounds in the present invention may include the prodrug form.

The patent applications and publications cited herein are incorporated by reference.

Biological property of the polypeptide compound (I) of the present invention

In order to show the usefulness of the polypeptide compound (I) of the present invention, the biological data of the representative compound is explained in the following.

Test (Antimicrobial activity) :

In vitro antimicrobial activity of the object compound of Example 5, 22, and 27 disclosed later was determined by MIC_s in mouse serum method as described below.

Test Method :

The MIC_s in mouse serum were determined by the microdilution method using ICR mouse serum buffered with 20 mM HEPES buffer (pH 7.3) as a test medium. Inoculum suspension of 10^6 cells/ml were prepared by a hemocytometric procedure and diluted to obtain an inoculum size of approximately 1.0×10^3 cells/ml. Microplates were incubated at 37°C for 24 hours in 5% CO_2. The MIC_s were defined as the lowest concentrations at which no visible growth was observed.

Test Result : MIC (μg/ml)

Test compound \ Test organism	Candida albicans FP-633
The object compound of Example 5	< 0.3
The object compound of Example 22	< 0.3
The object compound of Example 27	< 0.3

From the test result, it is realized that the object polypeptide compound (I) of the present invention has an antimicrobial activity (especially, antifungal activity).

The pharmaceutical composition of the present invention can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains the object polypeptide compound (I) or a pharmaceutically acceptable salt thereof, as an active ingredient in admixture with an organic or inorganic carrier or excipient which is suitable for rectal; pulmonary (nasal or buccal inhalation); ocular; external (topical); oral administration; parenteral (including subcutaneous, intravenous and intramuscular) administrations; insufflation (including aerosols from metered dose inhalator); nebulizer; or dry powder inhalator.

The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers in a solid form such as granules, tablets, dragees, pellets, troches, capsules, or suppositories; creams; ointments; aerosols; powders for insufflation; in a liquid form such as solutions, emulsions, or suspensions for injection; ingestion; eye drops; and any other form suitable for use. And, if necessary, there may be included in the

above preparation auxiliary substance such as stabilizing, thickening, wetting, emulsifying and coloring agents; perfumes or buffer; or any other commonly may be used as additives.

The object polypeptide compound (I) or a pharmaceutically acceptable salt thereof is/are included in the pharmaceutical composition in an amount sufficient to produce the desired antimicrobial effect upon the process or condition of diseases.

For applying the composition to human, it is preferable to apply it by intravenous, intramuscular, pulmonary, oral administration, eye drop administration or insufflation. While the dosage of therapeutically effective amount of the object polypeptide compound (I) varies from and also depends upon the age and condition of each individual patient to be treated, in the case of intravenous administration, a daily dose of 0.01-20 mg of the object polypeptide compound (I) per kg weight of human being in the case of intramuscular administration, a daily dose of 0.1-20 mg of the object polypeptide compound (I) per kg weight of human being, in case of oral administration, a daily dose of 0.5-50 mg of the object polypeptide compound (I) per kg weight of human being is generally given for treating or preventing infectious diseases.

Especially in case of the treatment of prevention of Pneumocystis carinii infection, the followings are to be noted.

For administration by inhalation, the compounds of the present invetion are conveniently delivered in the form of an aerosol spray presentation from pressurized as powders which may be formulated and the powder compositions may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery system for inhalation is a metered dose inhalation aerosol, which may be formulated as a suspension or solution of compound in suitable propellants such as

fluorocarbons or hydrocarbons.

Because of desirability to directly treat lung and bronchi, aerosol administration is a preferred method of administration. Insufflation is also a desirable method, especially where infection may have spread to ears and other body cavities.

Alternatively, parenteral administration may be employed using drip intravenous administration.

The following Preparations and Examples are given for the purpose of illustrating the present invention in more detail.

The Starting Compounds used and the Object Compounds obtained in the following Preparations 1 to 23 are given in the table as below, in which the formulas of the starting compounds are in the upper column and the formulas of the object compounds are in the lower column, respectively.

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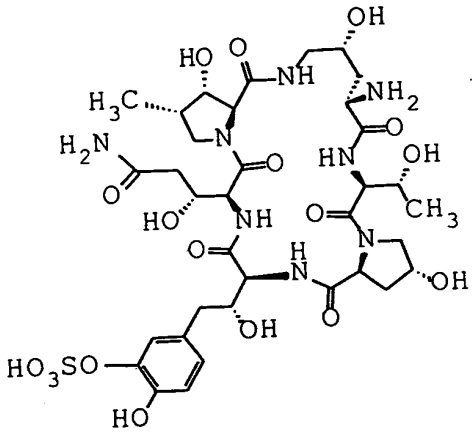
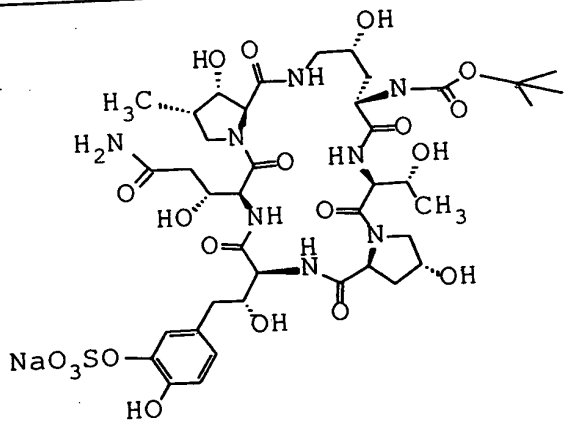
1	
	

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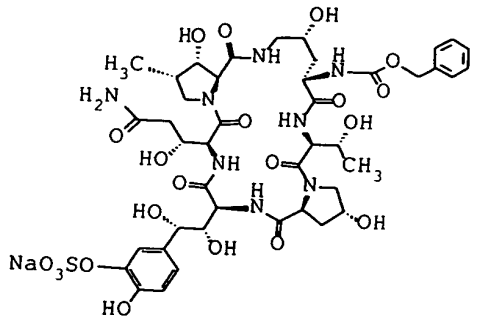
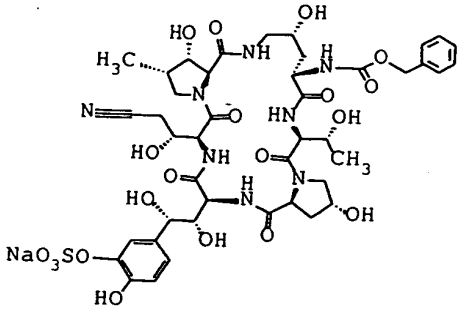
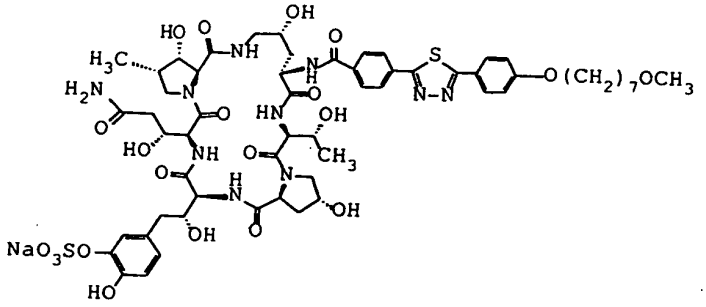
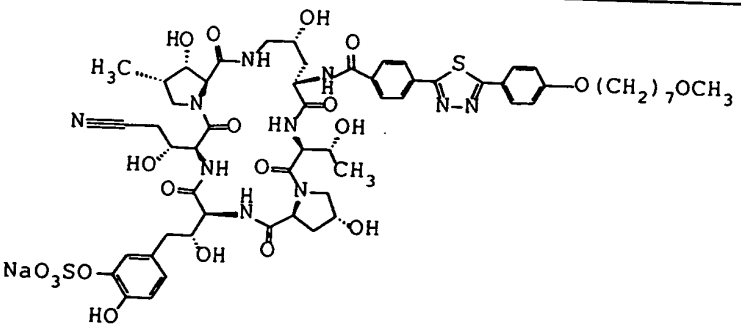
2	
	
3	
	

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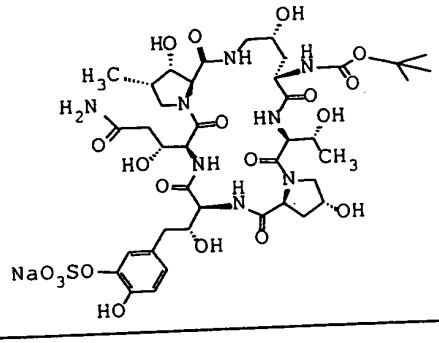
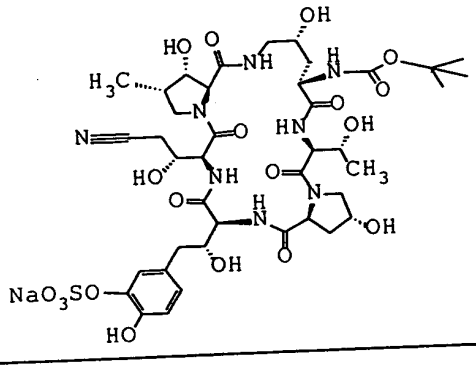
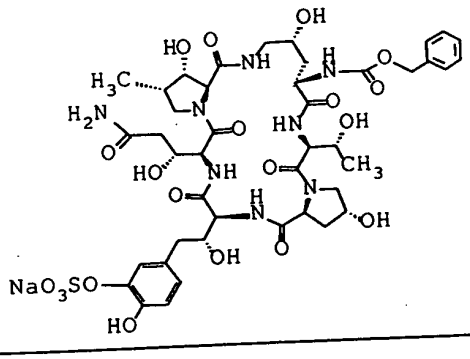
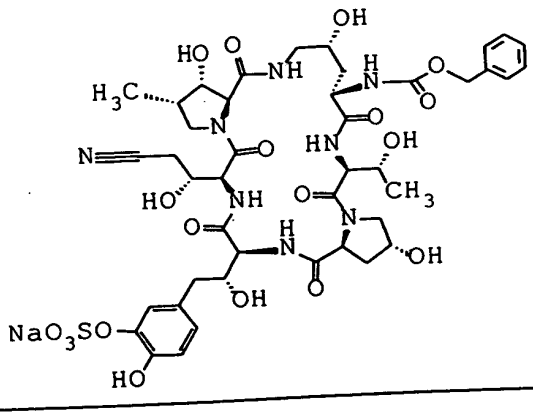
4	
	
5	
	

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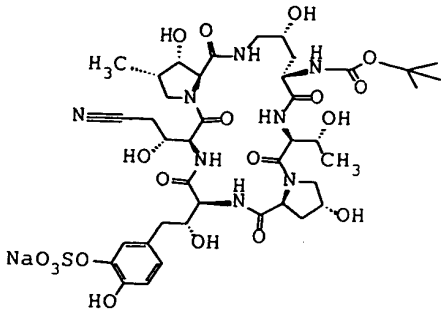
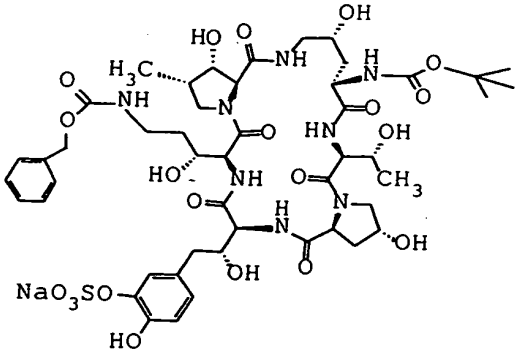
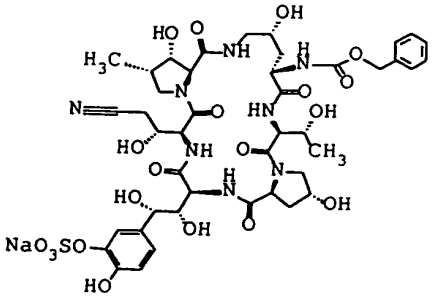
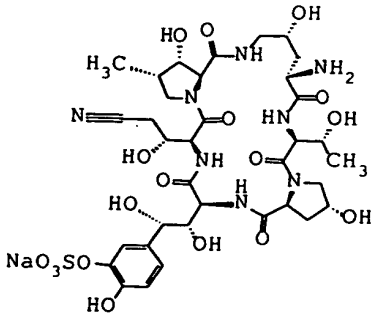
6	
	
7	
	

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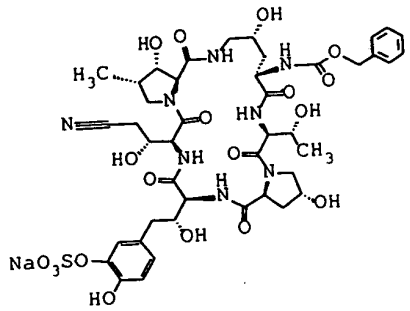
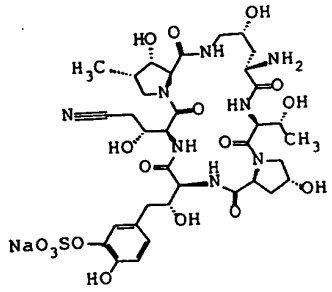
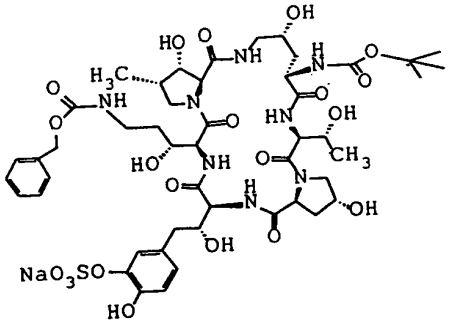
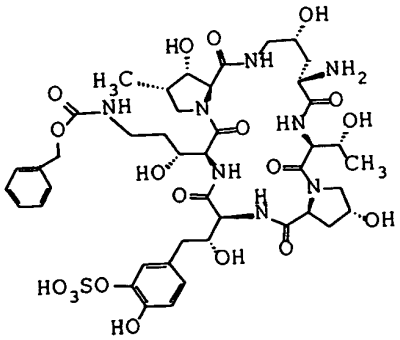
8	
	
9	
	

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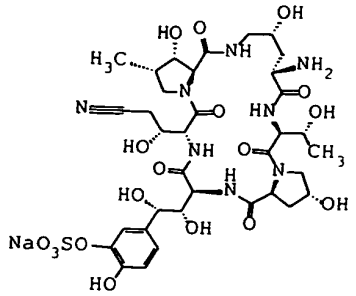
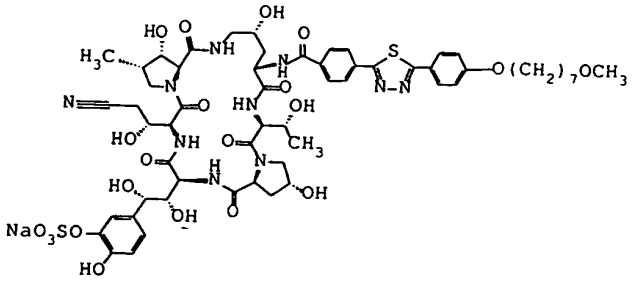
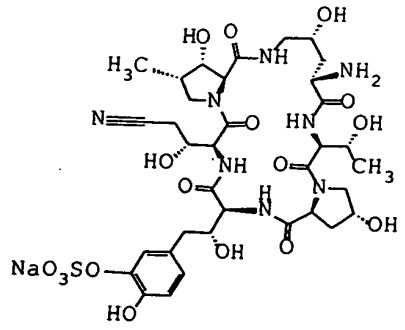
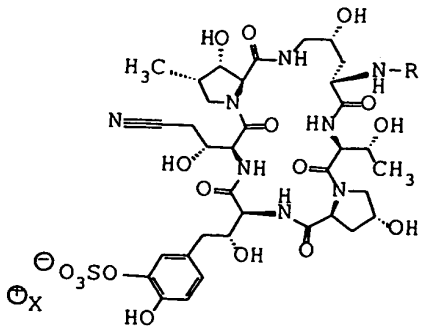
10	
	
11 ( ) 21	
	

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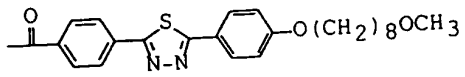
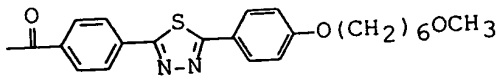
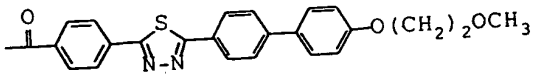
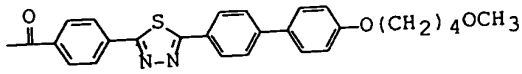
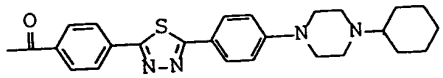
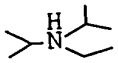
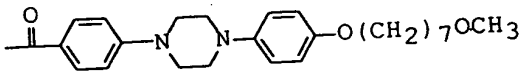
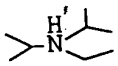
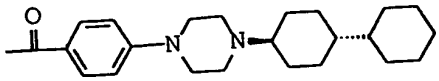
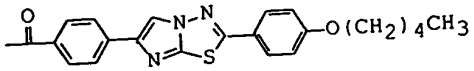
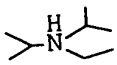
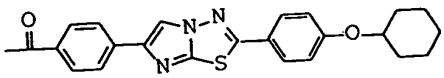
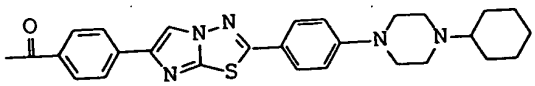
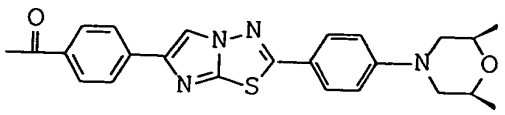
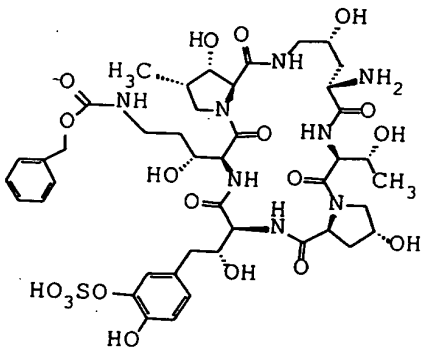
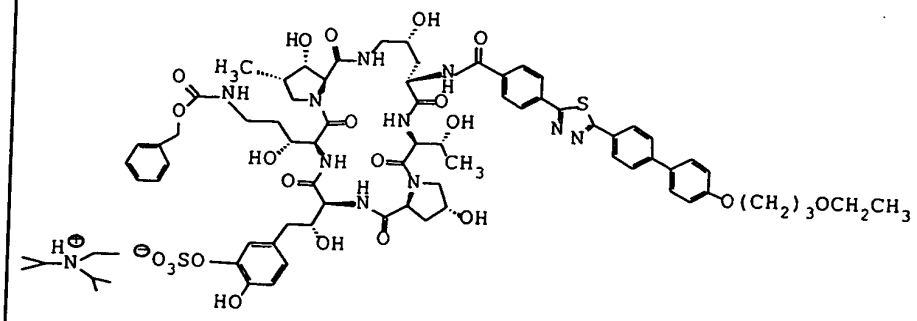
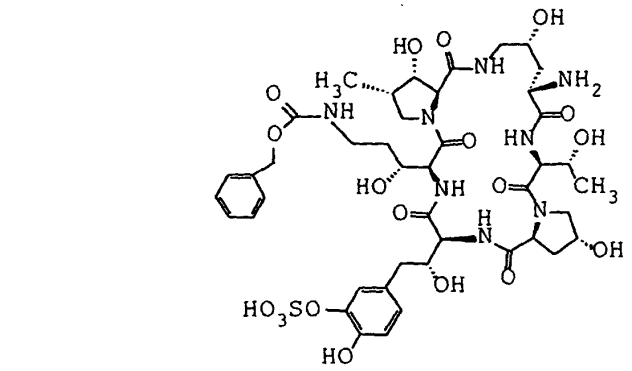
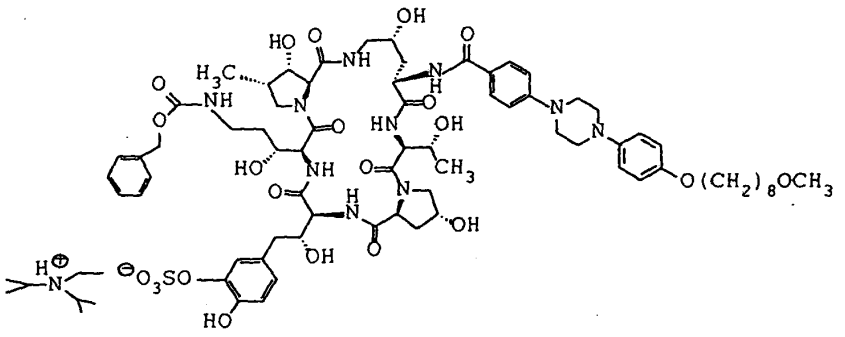
11		Na
12		Na
13		Na
14		Na
15		
16		
17		Na
18		
19		Na
20		Na

Preparation No.	R	X
21		Na

5

Preparation No.	Formula
22	
	

Preparation No.	Formula
23	
	

Preparation 1

A solution of Starting compound (20 g) in 1,4-dioxane (100 ml) was treated with a solution of 1N-sodium hydroxide (44.2 ml), diluted to 100 ml with water, and to the stirred mixture was added a solution of di-tert-butyldicarbonate (9.2 g) in 1,4-dioxane (50 ml) and then stirred for 2 hours at room temperature. 500 ml of pH 6.86 phosphate buffer and 100 ml ethyl acetate were added and the mixture was stirred and the organic layer discarded. The aqueous layer was adjusted to pH 7.0 with 1N-hydrochloric acid then evaporated to remove organic solvent, filtered, and purified by ODS column chromatography eluting with aqueous methanol (5-15%). Object compound containing fractions were pooled, evaporated, and lyophilized to give Object compound (19.61 g) as an amorphous

white powder.

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.8Hz), 1.07 (3H, d, J=5.5Hz), 1.34 (9H, s), 1.40-2.50 (9H, m), 2.80-3.0 (1H, m), 3.4-4.5 (15H, m), 4.70-5.40 (8H, m), 6.60-7.05 (6H, m), 7.25-8.00 (5H, m), 8.71 (1H, s)

MASS (m/z) : 1003.3 (M^+-H)

Preparation 2

A mixture of Starting compound (500 mg), N,N-dimethylformamide (5 ml) and synthetic A-4 zeolite (500 mg, Wako Chemical) was treated with diisopropyl ethylamine (66 mg), followed by methanesulfonyl chloride (58.5 mg) dropwise. After 1 hour at room temperature, further diisopropyl ethylamine (66 mg) and methanesulfonyl chloride (58.5 mg) were added. After 1.5 hours, additional diisopropylamine (66 mg) and methanesulfonyl chloride (58.5 mg) were added. After 1.5 hours, the mixture was filtered and the filtrate was poured into ethyl acetate. The precipitate was collected, washed with ethyl acetate and dried. The powder was dissolved in saturated sodium hydrogen carbonate solution then purified by ODS column chromatography (Daisogel SP-120 ODS Daiso) eluting with aqueous methanol (5-12.5%). Object compound-containing fractions were pooled, evaporated to remove methanol, and lyophilized to give Object compound (210 mg) as an amorphous white powder.

IR (KBr) : 2258.2, 1664.3, 1629.6, 1529.3, 1517.7, 1446.4, 1268.9 cm^{-1}

NMR (DMSO-d_6, δ) : 0.94 (3H, d, J=6.7Hz), 1.07 (3H, d, J=5Hz), 1.40-3.00 (9H, m), 3.10-4.50 (15H, m), 4.50-5.30 (10H, m), 5.66-5.69 (1H, m), 6.73 (1H, d, J=8.2Hz), 6.82 (1H, d, J=8Hz), 7.05 (1H, d, J=1.7Hz), 7.33 (5H, s), 7.20-7.50 (3H, m), 7.6-7.7 (1H, m), 8.27 (1H, d, J=8.3Hz), 8.84 (1H, s)

MASS (m/z) : 1081.3 ($M+Na^+$)

Elemental Analysis Calcd. for $C_{43}H_{55}N_8O_{20}SNa \cdot 6H_2O$:
C 44.25, H 5.79, N 9.60

Found : C 44.30, H 5.79, N 9.48

The following compounds [Preparations 3 to 5] were obtained according to a similar manner to that of Preparation 2.

Preparation 3

IR (KBr) : 2256.3, 1631.5, 1538.9, 1513.8, 1442.5, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.7Hz), 1.12 (3H, d, J=5.5Hz), 1.20-1.60 (8H, m), 1.65-3.05 (13H, m), 3.21 (3H, s), 3.30 (2H, t, J=6.4Hz), 3.45-4.57 (16H, m), 4.70-5.30 (7H, m), 5.87 (1H, d, J=6.1Hz), 6.72 (1H, d, J=8.2Hz), 6.76-6.81 (1H, m), 6.98 (1H, d, J=1.3Hz), 7.13 (2H, d, J=8.8Hz), 7.40-7.53 (2H, m), 7.79 (1H, br s), 7.98 (2H, d, J=8.7Hz), 8.10 (4H, s), 8.34 (1H, d, J=7.9Hz), 8.72 (1H, d, J=5.7Hz), 8.73 (1H, s)

MASS (m/z) : 1293.4 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{58}H_{73}N_{10}O_{20}SNa \cdot 6H_2O$:
C 48.87, H 6.01, N 9.83
Found : C 48.69, H 6.09, N 9.70

Preparation 4

IR (KBr) : 2256.3, 1666.2, 1631.5, 1535.1, 1515.8, 1448.3, 1442.5, 1272.8, 1251.6, 1166.7, 1083.8, 1047.2 cm^{-1}

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.7Hz), 1.09 (3H, d, J=5.1Hz), 1.35 (9H, s), 1.50-3.00 (9H, m), 3.10-4.50 (17H, m), 4.65-5.00 (5H, m), 5.15-5.17 (2H, m), 5.70-5.90 (1H, m), 6.68-6.78 (2H, m), 6.86-6.96 (2H, m), 7.32 (1H, d, J=8Hz), 7.40-7.50 (1H, m), 7.70-7.80 (1H, m), 8.30-8.40 (1H, m), 8.72 (1H, s)

MASS (m/z) : 985.3 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{40}H_{58}N_8O_{19}SNa \cdot 9H_2O$:
C 41.02, H 6.45, N 9.57

Found : C 41.35, H 6.42, N 9.61

Preparation 5

IR (KBr) : 2256.3, 1668.1, 1648.8, 1631.5, 1538.9, 1513.8, 1454.1, 1267.0 cm^{-1}

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.8Hz), 1.07 (3H, d, J=5.2Hz), 1.5-2.9 (10H, m), 3.2-4.5 (15H, m), 4.7-5.2 (9H, m), 5.7-5.8 (1H, m), 6.60-6.78 (2H, m), 6.96 (1H, br s), 7.33 (5H, s), 7.2-7.5 (3H, m), 7.7-7.8 (1H, m), 8.3 (1H, d, J=7.5Hz), 8.73 (1H, br s)

MASS (m/z) : 1065.2 ($M+Na^+$)

Elemental Analysis Calcd. for $C_{43}H_{55}N_8O_{19}SNa \cdot 7H_2O$:
C 44.18, H 5.95, N 9.58
Found : C 44.21, H 5.82, N 9.54

Preparation 6

A solution of Starting compound (2.0 g) in methanol (100 ml) - water (20 ml) was treated with cobalt(II) chloride hexahydrate (1.89 g) and then stirred to give a pink solution. Sodium borohydride (1.5 g) was then added portionwise and then stirred for 1 hour at room temperature. The reaction mixture was filtered through a bed of celite, washing with methanol (100 ml) - water (30 ml) solution. The ice-cooled filtrate was then treated dropwise with a solution of benzyloxy carbonyl chloride (Z-chloride) (0.34 ml) in tetrahydrofuran (5 ml) and stirred for 1 hour at the same temperature. Ethyl acetate (50 ml) was added followed by water (200 ml) and after stirring ~ 5 minutes, the separated organic layer was discarded. The aqueous layer was adjusted to pH 8.8 and evaporated to remove organic solvent and then purified by ODS column chromatography, eluting with aqueous acetonitrile (10-30%). Object compound containing fractions were pooled, evaporated, and lyophilized to give Object compound (1.61 g) as an amorphous white powder.

IR (KBr) : 1666.2, 1631.5, 1517.7, 1444.4, 1267.0 cm^{-1}

NMR (DMSO-d_6, δ) : 0.94 (3H, d, J=6.7Hz), 1.00-1.15 (3H, m), 1.33 (9H, s), 1.35-2.10 (6H, m), 2.10-2.50 (4H, m), 2.80-3.30 (4H, m), 3.60-4.55 (12H, m), 4.60-4.90 (2H, m), 4.99 (2H, s), 4.50-5.30 (4H, m), 6.60-7.10 (4H, m), 7.33 (5H, s), 7.35-7.90 (3H, m), 8.72 (1H, br s)

MASS (m/z) : 1123.3 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{48}H_{67}N_8O_{21}SNa \cdot 6H_2O$:
C 45.93, H 6.34, N 8.93
Found : C 45.68, H 6.33, N 8.82

Preparation 7

A solution of Starting compound (2.0 g) in methanol (30 ml) was treated with wet 10% palladium on carbon (1.5 g) and exposed to one atmosphere of hydrogen gas via balloon. After 5.5 hours, water (4 ml) was added and hydrogenation continued for a further 30 minutes. Methanol (100 ml) was added and the catalyst removed by filtration. The solution was concentrated in vacuo to remove methanol and the aqueous residue lyophilized to give Object compound (1.69 g) as a pink colored amorphous powder.

IR (KBr) : 2256.3, 1648.8, 1631.5, 1538.9, 1515.8, 1440.6, 1083.8, 1047.2 cm^{-1}

NMR (DMSO-d_6, δ) : 0.94 (3H, d, J=6.7Hz), 1.09 (3H, d, J=5.9Hz), 1.7-2.8 (10H, m), 3.0-4.5 (19H, m), 4.6-5.3 (6H, m), 5.85-6.0 (1H, m), 6.72 (1H, d, J=8.2Hz), 6.82 (1H, dd, J=1.8 and 8.4Hz), 7.06 (1H, d, J=1.7Hz), 7.32 (1H, d, J=8.9Hz), 7.44 (1H, d, J=9.1Hz), 7.6-7.8 (2H, m), 7.8-8.0 (1H, br s)

MASS (m/z) : 901.2 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{35}H_{49}N_8O_{18}SNa \cdot 6H_2O$:
C 40.70, H 5.95, N 10.85
Found : C 40.60, H 5.94, N 10.71

The following compound was obtained according to a similar manner to that of Preparation 7.

Preparation 8

IR (KBr) : 2256.3, 1648.8, 1631.5, 1538.9, 1513.8, 1267.0, 1083.8, 1047.2 cm^{-1}

NMR (DMSO-d_6, δ) : 0.96 (3H, d, J=6.7Hz), 1.08 (3H, d, J=5.9Hz), 1.7-2.1 (2H, m), 2.1-2.9 (7H, m), 3.1-4.6 (16H, m), 4.7-5.4 (6H, m), 6.1 (1H, br s), 6.70 (1H, d, J=8.2Hz), 6.75 (1H, d, J=8.2Hz), 6.96 (1H, br s), 7.2-7.55 (2H, m), 7.6-7.9 (2H, m)

MASS (m/z) : 885.3 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{35}H_{49}N_8O_{17}SNa \cdot 6H_2O$: C 41.34, H 6.05, N 11.02

Found : C 41.58, H 5.99, N 10.94

Preparation 9

A suspension of Starting compound (1.6 g) in dichloromethane (41 ml) was stirred with cooling at 5°C and treated with triethylsilane (1.1 ml), followed by trifluoroacetic acid (5.3 ml) dropwise over 30 minutes. After warming to room temperature, the clear solution was stirred for 2 hours, then poured into 450 ml of pH 6.86 phosphate buffer and adjusted to pH 8.5 with 4N-sodium hydroxide solution. Organic solvent was removed by evaporation and the remaining aqueous solution purified by ODS column chromatography, eluting with aqueous acetonitrile (5-20%). Object compound-containing fractions were pooled, evaporated, and lyophilized to give Object compound (1.25 g) as an amorphous white powder.

IR (KBr) : 1633.4, 1537.0, 1517.7, 1440.6, 1267.0 cm^{-1}

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.7Hz), 1.12 (3H, d, J=5.8Hz), 1.27 (2H, d, J=6.6Hz), 1.28-1.70 (2H, m), 1.75-2.45 (4H, m), 2.65-3.30 (5H, m), 3.50-4.50 (11H, m), 4.60-4.90 (2H, m), 5.00 (2H, s), 5.05-5.40 (5H, m), 6.70 (2H, d, J=8.2Hz), 6.76 (2H, d, J=8.2Hz), 6.96 (1H, s), 7.00-7.15 (1H, m), 7.34 (5H, s), 7.40-7.95 (3H, m), 8.60-8.90 (1H, m)

MASS (m/z) : 1023.3 ($M-H^+$)

Elemental Analysis Calcd. for $C_{43}H_{60}N_8O_{19}S \cdot 6H_2O$:
C 45.58, H 6.40, N 9.89
Found : C 45.49, H 6.24, N 9.70

Preparation 10

A solution of Starting compound (3 g) in N,N-dimethylformamide (60 ml) was treated with 4-[5-[4-(7-methoxy-n-heptyloxy)phenyl]-1,3,4-thiadiazol-2-yl]benzoic acid benzotriazol-1-yl ester (2.65 g) and diisopropylethylamine (0.564 ml) and stirred for 4 hours 20 minutes at room temperature. Ethyl acetate (1 l)was added and the resulting precipitate collected, washed with isopropyl ether, and dried to give Object compound (5.62 g) as a crude powder that was used directly in the next step without purification.

The following compounds [Preparations 11 to 17] were obtained according to a similar manner to that of Preparation 10.

Preparation 11

The object comopund was used directly in the next step without purification.

Preparation 12

The object comopund was used directly in the next step without purification.

Preparation 13

MASS (m/z) : 1299.3 ($M-Na^+$)

Preparation 14

The object comopund was used directly in the next step without purification.

Preparation 15

The object comopund was used directly in the next step without purification.

Preparation 16

The object comopund was used directly in the next step without purification.

Preparation 17

MASS (m/z) : 1237.3 ($M-Na^+$)

Preparation 18

A mixture of 4-[2-(4-pentyloxyphenyl)imidazo[2,1-b]-[1,3,4]thiadiazol-6-yl]benzoic acid (1.44 g), 1-hydroxybenzotriazole (714 mg), diisopropyl ethylamine (0.58 ml) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (810 mg) in N,N-dimethylformamide (50 ml) was stirred 6 hours at room temperature, then treated with Starting compound (2 g) and stirred overnight. Additional N,N-dimethylformamide (20 ml) was added and stirring continued for a further 5.5 hours. The clear solution was poured into ethyl acetate (1 l) and the precipitate collected and washed with isopropyl ether and dried to give crude Object compound (3.58 g), which was used directly in the next step.

The following compounds [Preparations 19 and 20] were obtained according to a similar manner to that of Preparation 18.

Preparation 19

MASS (m/z) : 1286.3 ($M-Na^+$)

Preparation 20

MASS (m/z) : 1354.4 ($M-Na^+$)

The following compound was obtained according to a

similar manner to that of Preparation 18.

Preparation 21

IR (KBr) : 1648.8, 1631.5, 1537.0, 1513.8, 1456.0 cm^{-1}

NMR (DMSO-d_6, δ) : 0.96 (3H, d, J=7Hz), 1.10 (3H, d, J=5.6Hz), 1.18 (6H, d, J=6Hz), 1.4-5.3 (38H, m), 5.88 (1H, d, J=6Hz), 6.71 (1H, d, J=8Hz), 6.75-6.80 (1H, m), 6.97 (1H, br s), 7.12 (2H, d, J=9Hz), 7.42 (1H, d, J=7.6Hz), 7.50 (1H, d, J=9Hz), 7.78 (2H, d, J=8.8Hz), 7.7-8.0 (1H, br s), 7.96 (4H, s), 8.32 (1H, d, J=8Hz), 8.50 (1H, d, J=7.1Hz), 8.72 (1H, s), 8.79 (1H, s)

MASS (m/z) : 1301.4 ($M-Na^+$)

The following compounds [Preparations 22 to 23] were obtained according to a similar manner to that of Preparation 10.

Preparation 22

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.6Hz), 6.67 (1H, d, J=6.9Hz), 6.73-6.75 (1H, m), 6.96 (1H, br s), 7.07 (2H, d, J=8.8Hz), 7.32 (5H, s), 7.73 (2H, d, J=8.7Hz), 7.87 (2H, d, J=8.5Hz), 8.06-8.14 (6H, m), 8.72 (1H, s), 8.80 (1H, d, J=7.1Hz)

MASS (m/z) : 1465.5 ($M-Na^+$)

Preparation 23

The object comopund was used directly in the next step without purification.

Preparation 24

To a solution of 1-N-t-butyloxycarbonyl-4-hydroxypiperidine (5.0 g) in dimethylformamide (DMF) (25 ml) was portionwise added sodium hydride (60% in oil) (1.29 g) with stirring under ice-cooling. The mixture was successively stirred at ambient temperature for 30 minutes, stirred at 60°C for 1 hour and cooled with an ice bath. To the reaction mixture was added 1,5-dibromopentane (6.72 ml), and the mixture was stirred at ambient temperature for 3 hours. The reaction solution was poured into water (100 ml) and extracted twice with a mixture of ethyl acetate (80 ml) and n-hexane (30 ml). The extract was washed with saturated aqueous sodium chloride, dried over magnesium sulfate and evaporated in vacuo. The resulting residue was chromatographed on silica gel (200 ml) eluting with a mixture of n-hexane and ethyl acetate (5:1 v/v). The fractions containing the desired compound were collected and evaporated under reduced pressure to give 4-(5-bromopentyloxy)-1-N-t-butoxycarbonylpiperidine (2.44 g).

NMR ($CDCl_3$, δ): 1.46 (9H, s), 1.50-1.70 (6H, m), 1.70-1.96 (4H, m), 3.00-3.15 (2H, m), 3.35-3.50 (5H, m), 3.70-3.90 (2H, m)

APCI MASS (m/z): 250 (M^+-101)

Preparation 25

To a solution of 4-(5-bromopentyloxy)-1-N-t-butoxycarbonylpiperidine (2.44 g) in methanol (13 ml) was added 28% sodium methoxide methanol solution (14.2 ml), and the mixture was stirred under reflux for 4 hours. The reaction mixture was evaporated in vacuo. The resulting residue was chromatographed on silica gel (250 ml) eluting with a mixture of n-hexane and ethyl acetate (5:1 v/v). The fractions containing the object compound were collected and evaporated under reduced pressure to give 4-(5-methoxypentyloxy)-1-N-t-butoxycarbonylpiperidine (1.97 g).

NMR ($CDCl_3$, δ): 1.45 (9H, s), 1.45-1.95 (10H, m), 3.03 (1H, dd, J=3.47 and 9.20Hz), 3.10 (1H, dd, J=3.47 and 9.20Hz), 3.44 (3H, s), 3.34-3.50 (5H, m), 3.70-3.85 (2H, m)

APCI MASS (m/z): 202 (M^+-101)

Preparation 26

To a solution of 4-(5-methoxypentyloxy)-1-N-t-butoxycarbonylpiperidine (1.97 g) in ethyl acetate (20 ml) was added 4N-hydrogen chloride ethyl acetate solution (16.3 ml), and the mixture was stirred at ambient temperature for 2 hours. The reaction mixture was evaporated in vacuo. The resulting residue was dissolved in a mixture of dichloromethane and methanol (10:1; 50 ml:5 ml). To this solution was added 1N-sodium hydroxide (5 ml) with stirring. The organic layer was separated and evaporated under reduced pressure to give 4-(5-methoxypentyloxy)piperidine (0.62 g).

NMR ($CDCl_3$, δ): 1.25-1.50 (2H, s), 1.50-1.75 (6H, m), 1.90-2.10 (2H, m), 2.70-2.90 (2H, m), 2.95-3.20 (2H, m), 3.33 (3H, s), 3.35-3.50 (5H, m)

APCI MASS (m/z): 202 (M^+)

Preparation 27

A solution of 4-fluorobenzonitrile (0.38 g), 4-(5-methoxypentyloxy)piperidine (0.62 g) and potassium carbonate (0.87 g) in DMF (8 ml) was stirred at 90-95°C for 6 hours. The reaction mixture was poured into water (50 ml) and extracted twice with a mixture of ethyl acetate and n-hexane (50 ml:20 ml). The extracts were combined, washed with saturated aqueous sodium chloride, dried over magnesium sulfate and evaporated in vacuo. The resulting residue was chromatographed on silica gel (100 ml) eluting with a mixture of n-hexane and ethyl acetate (5:1 v/v - 2:1 v/v). The fractions containing the desired compound were collected and evaporated under reduced pressure

to give 4-(5-methoxypentyloxy)-N-(4-cyanophenyl)piperidine (294 mg).

NMR ($CDCl_3$, δ): 1.35-1.55 (2H, s), 1.55-1.75 (5H, m), 1.85-2.05 (2H, m), 3.13 (1H, dd, J=3.47 and 9.20Hz), 3.17 (1H, dd, J=3.47 and 9.20Hz), 3.33 (3H, s), 3.35-3.75 (8H, m), 6.85 (2H, d, J=9.01Hz), 7.47 (2H, d, J=8.96Hz)

APCI MASS (m/z): 303 (M^+)

Preparation 28

A solution of 4-(5-methoxypentyloxy)-N-(4-cyanophenyl)piperidine (294 mg) and thiosemicarbazide (0.68 g) in toluene (20 ml) and trifluoroacetic acid (10 ml) was stirred at 60-65°C for 7 hours. After cooling, the reaction mixture was poured into a mixture of water (100 ml) and ethyl acetate (200 ml) and adjusted to pH 10 with 1N-sodium hydroxide. The mixture was dissolved in a mixture of THF (50 ml) and methanol (10 ml). The organic layer was separated, washed with saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate and evaporated in vacuo. The resulting precipitate was washed with isopropyl ether and dried in vacuo to give 2-amino-5-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]-1,3,4-thiadiazole (1.29 g).

NMR ($CDCl_3$+CD_3OD, δ): 1.30-1.50 (2H, m), 1.50-1.80 (6H, m), 1.90-2.10 (2H, m), 2.90-3.10 (2H, m), 3.34 (3H, s), 3.35-3.70 (7H, m), 6.93 (2H, d, J=8.91Hz), 7.63 (2H, d, J=8.83Hz)

APCI MASS (m/z): 377 (M^+)

Preparation 29

To a suspension of 2-amino-5-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]-1,3,4-thiadiazole (1.29 g) in ethanol (20 ml) was added ethyl 4-bromoacetylbenzoate (1.39 g) and stirred at reflux for 5 hours.

The reaction mixture was cooled and poured into diisopropyl ether (IPE) (60 ml). The resulting precipitate was collected by filtration and dried. To a suspension of the precipitate in xylene (40 ml) was added trifluoroacetic acid (4 ml), and the mixture was stirred at reflux (130°C) for 5 hours. The reaction mixture was cooled and poured into IPE (300 ml). The resulting precipitate was filtered and dried to give 4-[2-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid ethyl ester trifluroacetic acid salt (2.01 g).

NMR ($CDCl_3$, δ): 1.42 (3H, t, J=7.12Hz), 1.45-1.75 (6H, m), 1.85-2.10 (2H, m), 2.30-2.50 (2H, m), 3.36 (3H, s), 3.35-3.55 (5H, m), 3.60-3.80 (2H, m), 4.40 (2H, q, J=7.14Hz), 7.57 (2H, d, J=8.78Hz), 7.84 (2H, d, J=8.40Hz), 7.91 (2H, d, J=8.79Hz), 8.13 (1H, s)

ESI MASS (m/z): 549 (M^++1)

Preparation 30

To a solution of 4-[2-[4-[4-(5-methoxypentyloxy)-piperidin-1-yl]phenyl]imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid ethyl ester trifluroacetic acid salt (2.01 g) in a mixture of methanol (40 ml) and tetrahydrofuran (20 ml) was added 4N-NaOH (20 ml), and the mixture was refluxed for 6 hours. The reaction mixture was cooled, poured into water (200 ml) and adjusted to pH 2 with conc. HCl. The resulting precipitate was collected by filtration, washed in turn with water, isopropyl alcohol (30 ml) and IPE (50 ml) to give 4-[2-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid (1.28 g).

ESI MASS (m/z)(Negative): 519.2 (M^++1)

Preparation 31

To a solution of 4-[2-[4-[4-(5-methoxypentyloxy)-

piperidin-1-yl]phenyl]imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid (1.28 g) and 1-hydroxybenzotriazole (465 mg) in dichloromethane (50 ml) was added 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl) (943 mg), and the mixture was stirred overnight at ambient temperature. The reaction mixture was evaporated in vacuo. To the resulting precipitate was added water (50 ml) and filtrated. The precipitate was washed with water and IPE (50 ml) and dried under reduced pressure for 3 hours to give 4-[2-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid benzotriazol-1-yl ester (1.26 g).

IR (KBr): 1774.2, 1708.6, 1604.5, 1471.4 1365.4, 1230.4 cm^{-1}

NMR ($CDCl_3$, δ): 1.30-1.80 (8H, m), 1.85-2.10 (2H, m), 3.05-3.30 (2H, m), 3.33 (3H, s), 3.35-3.55 (4H, m), 3.55-3.75 (2H, m), 6.94 (2H, d, J=8.94Hz), 7.30-7.60 (3H, m), 7.73 (2H, d, J=8.79Hz), 8.00-8.20 (4H, m), 8.30 (2H, d, J=8.46Hz)

ESI MASS (m/z)(Positive): 660.1 (M^++Na)

The Starting Compounds used and the Object Compounds obtained in the following Examples 1 to 30 are given in the table as below, in which the formulas of the starting compounds are in the upper column, and the formulas of the object compounds are in the lower column, respectively.

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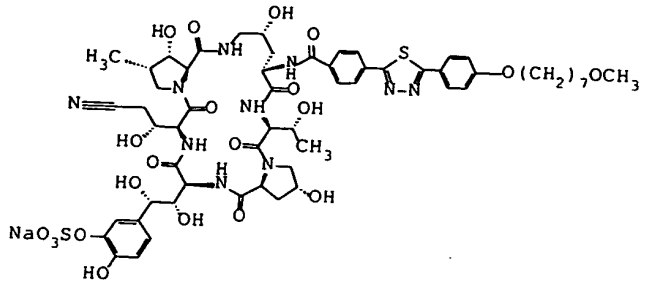
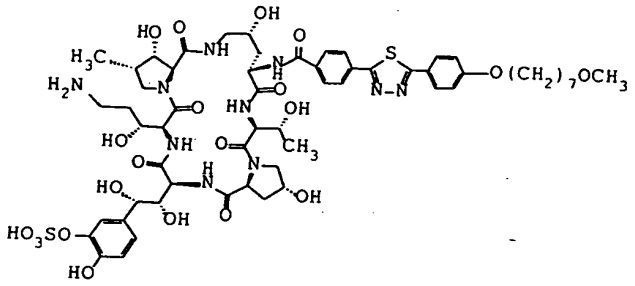
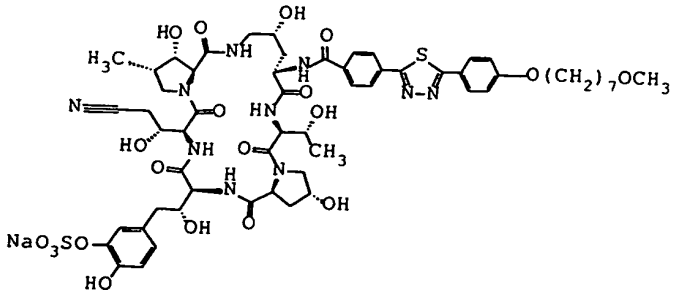
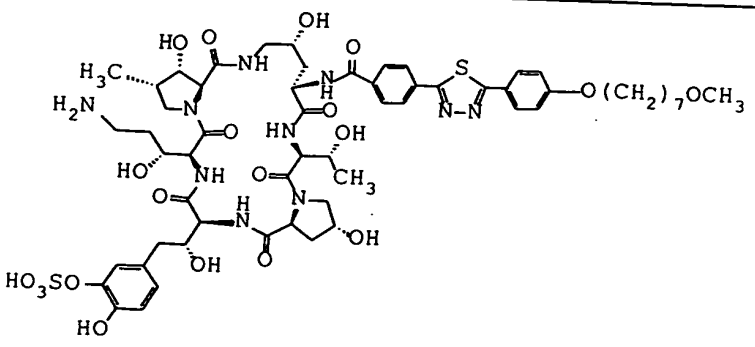
1	
	
2	
	

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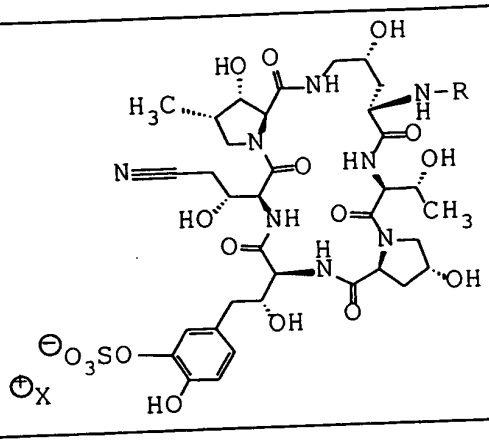
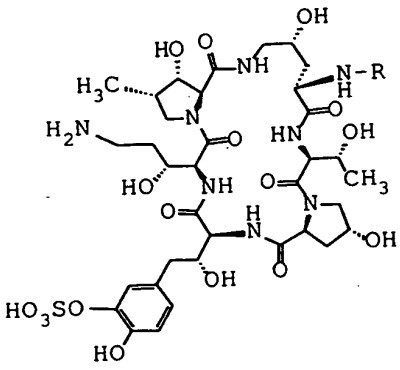
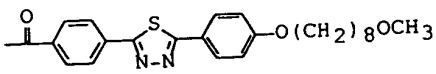
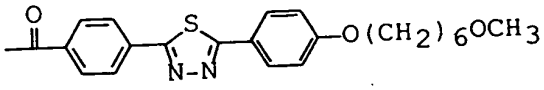
3 ~ 13	
	

Example No.	R	X
3		Na
4		Na

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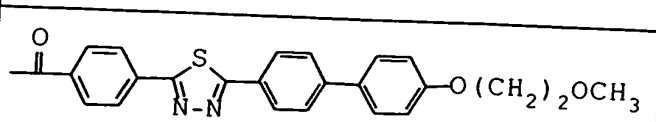
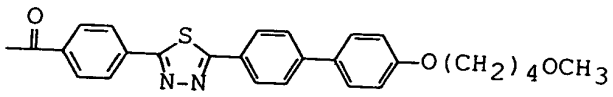
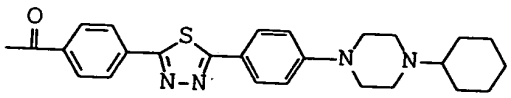
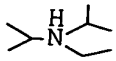
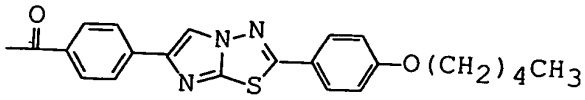
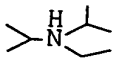
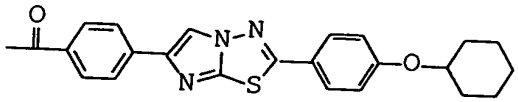
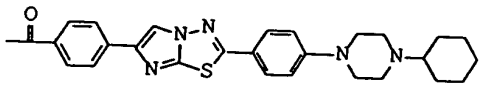
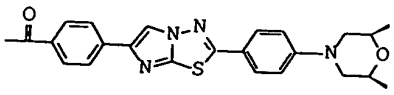
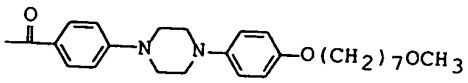
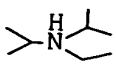
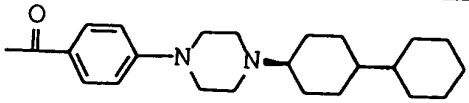
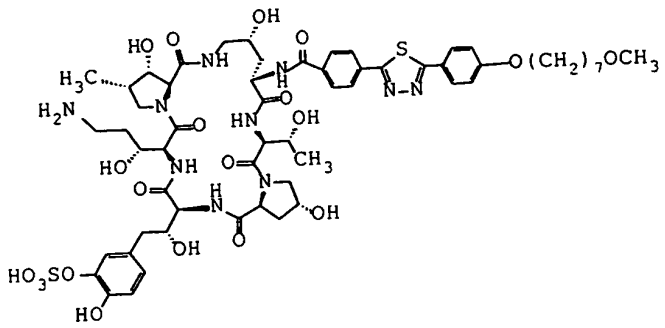
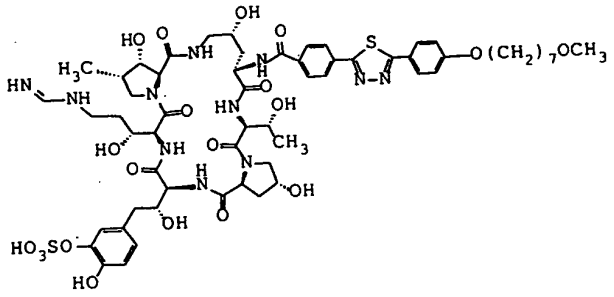
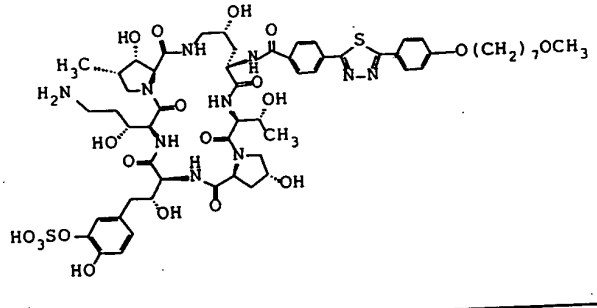
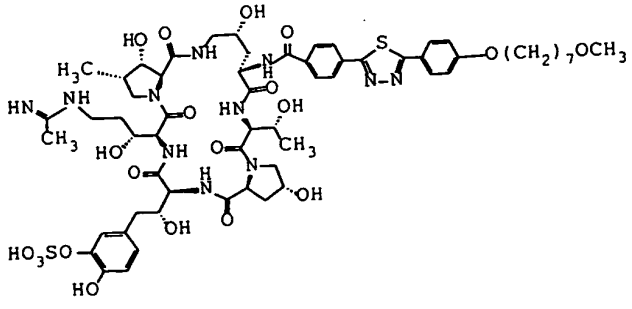
5		Na
6		Na
7		
8		
9		Na
10		Na
11		Na
12		
13		Na

Example No.	Formula
14	
	
15	
	

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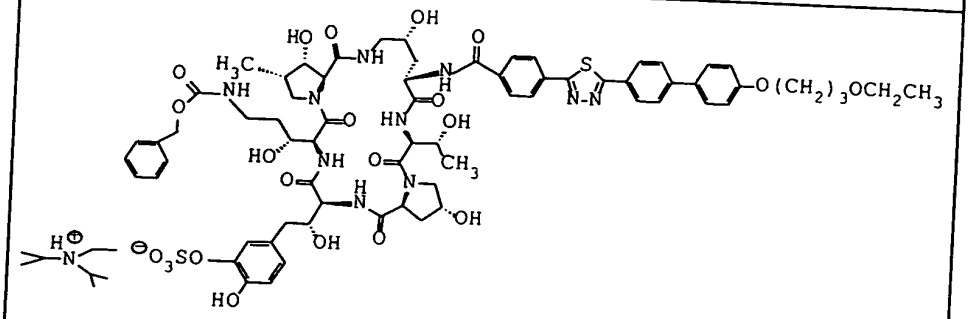
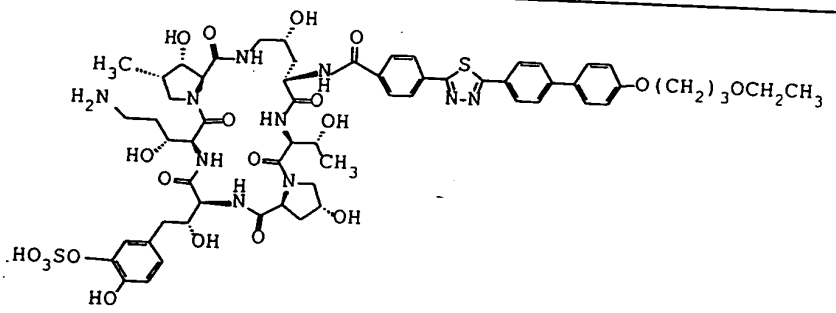
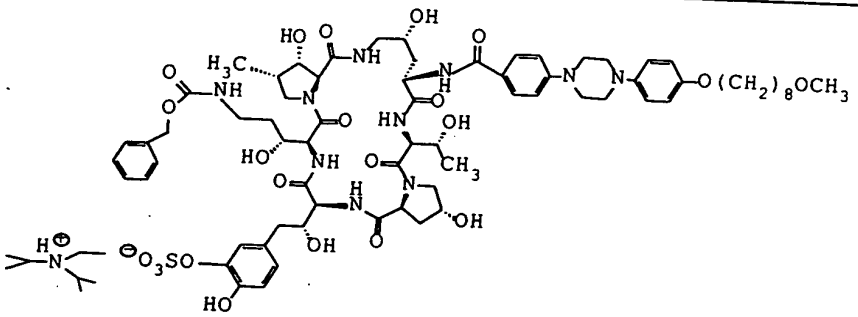
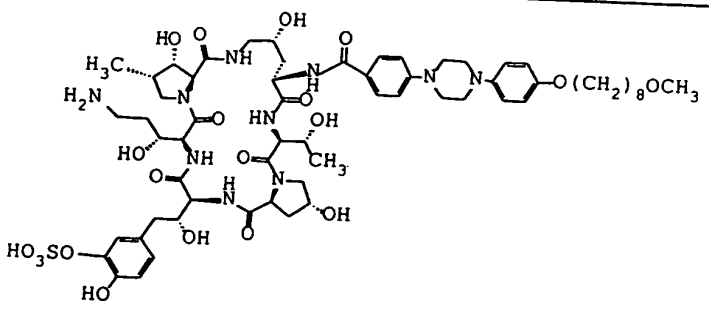
16	
	
17	
	

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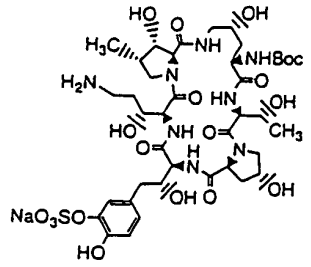
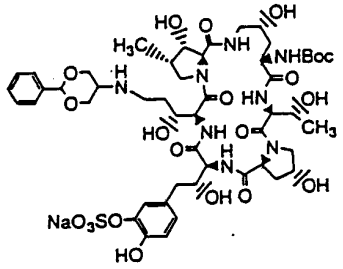
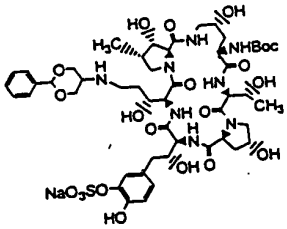
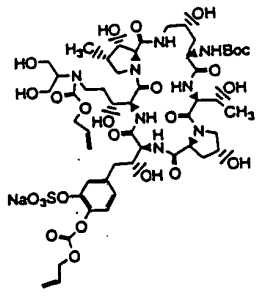
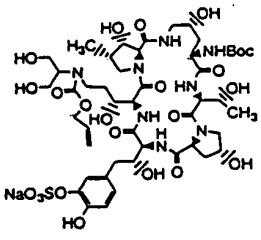
18	
	
19	
	major  minor 

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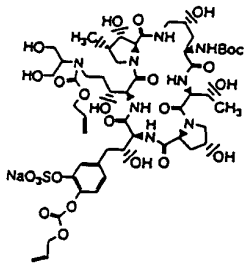
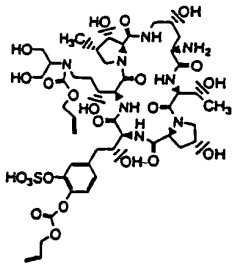
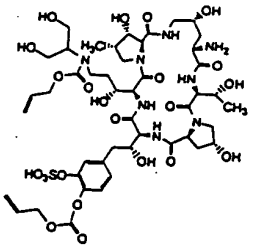
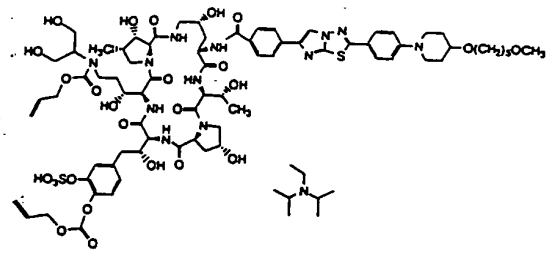
20	
	
21	
	

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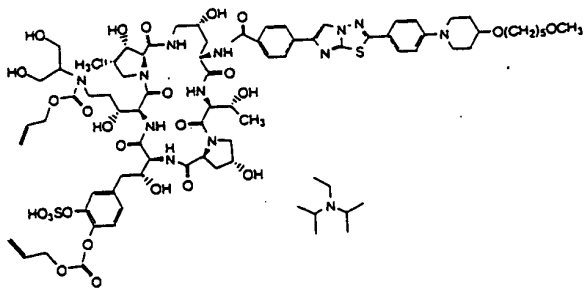
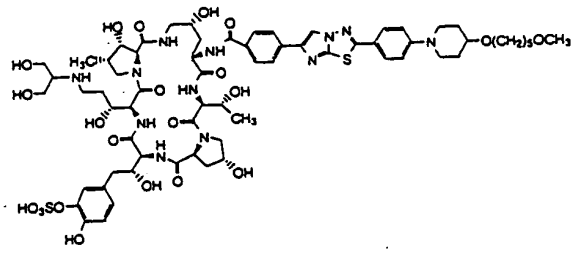
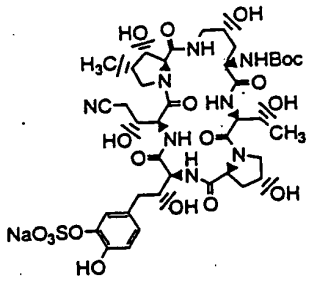
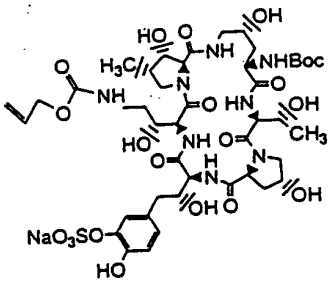
22	
	
23	
	

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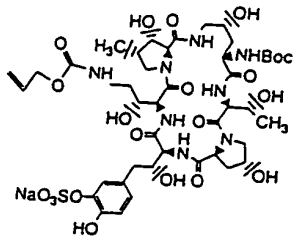
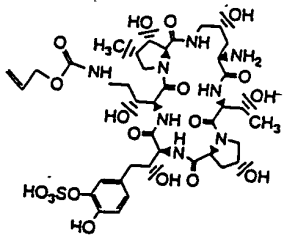
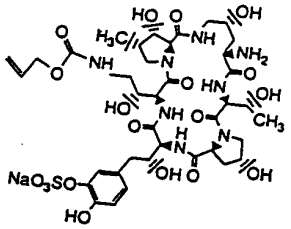
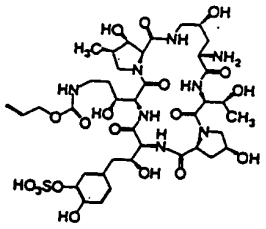
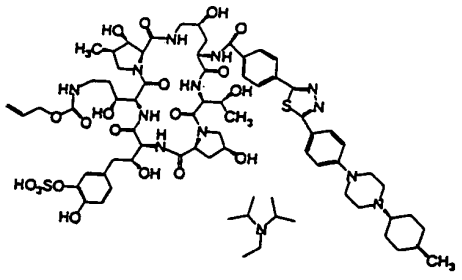
24	
	major  minor 
25	
	

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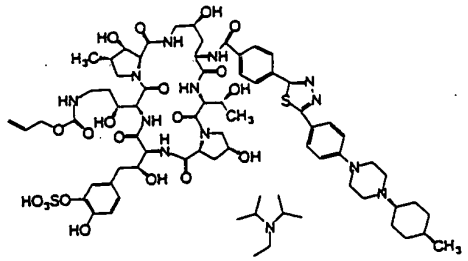
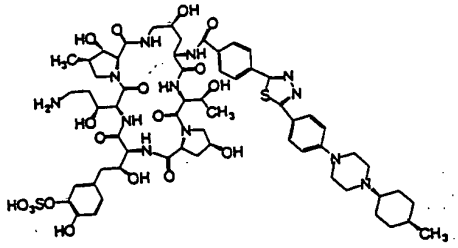
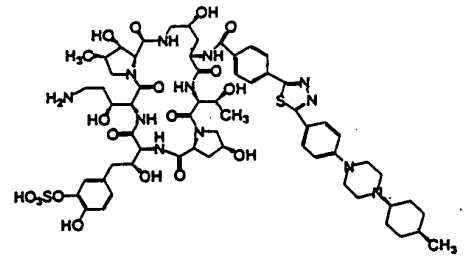
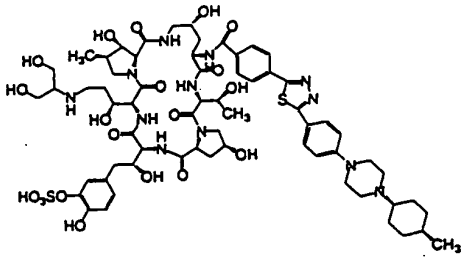
26	
	
27	
	

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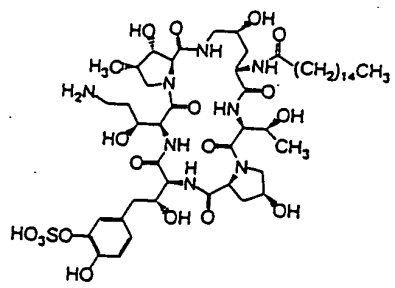
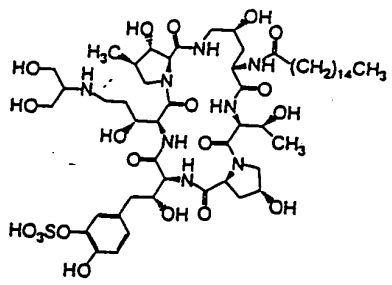
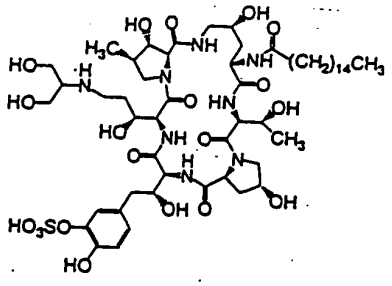
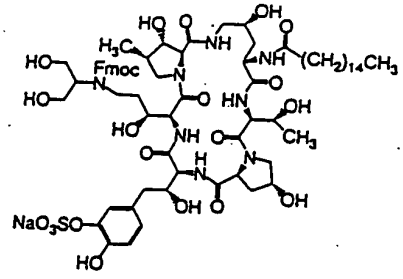
28	
	
29	
	

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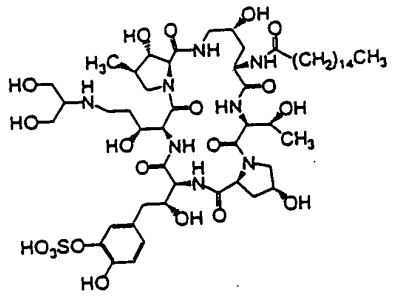
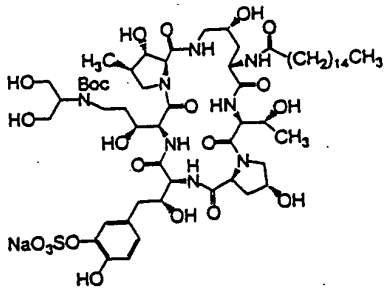
30	
	

Example 1

A solution of crude Starting compound (5.6 g) in methanol (168 ml) - water (336 ml) was treated with cobalt chloride hexahydrate (3.08 g) and the mixture stirred to give a pink colored solution. Sodium borohydride (2.46 g) was then added portionwise over 1 hour. Additional cobalt chloride (1.54 g) was added followed by sodium borohydride (1.23 g, portionwise). After a total reaction time of 2 hours 50% aqueous acetonitrile (600 ml) was added and insoluble material removed by filtration. The filtrate was evaporated to remove organic solvent and sufficient 1N-sodium hydroxide was added to the remaining aqueous layer to effect solution. This clear aqueous solution was then purified by ODS column chromatography eluting with aqueous acetonitrile. Object compounds-containing fractions were pooled, evaporated, and lyophilized to give Object compound (1.4 g) as an amorphous white powder.

IR (KBr) : 1658.5, 1635.3, 1546.6, 1529.3, 1517,7, 1444.4, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.96 (3H, d, J=6.8Hz), 1.10 (3H, d, J=6Hz), 1.30-1.60 (8H, m), 1.60-2.50 (15H, m), 3.21 (3H, s), 2.80-5.40 (29H, m), 6.74 (1H, d, J=8.2Hz), 6.80-6.85 (1H, m), 7.07 (1H, br s), 7.14 (2H, d, J=8.9Hz), 7.40-7.80 (4H, m), 7.97 (2H, d, J=8.8Hz), 8.09 (4H, ABq like, br m), 8.20-8.30 (1H, m), 8.80-8.90 (1H, m)

MASS (m/z) : 1313.3($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{78}N_{10}O_{21}S_2 \cdot 9H_2O$: C 47.34, H 6.16, N 9.52

Found : C 47.42, H 6.26, N 9.47

The following compounds [Examples 2 to 13] were obtained according to a similar manner to that of Example 1.

Example 2

IR (KBr) : 1648.8, 1631.5, 1538.9, 1515.8, 1442.5, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.98 (3H, d, J=6.7Hz), 1.24 (3H, d, J=5.6Hz), 1.40-1.60 (8H, m), 1.60-2.65 (15H, m), 2.80-5.50 (27H, m), 3.21 (3H, s), 3.30 (2H, t, J=6.3Hz), 6.72 (1H, d, J=8.1Hz), 6.78 (1H, dd, J=1.6 and 8.3Hz), 7.00 (1H, d, J=1.6Hz), 7.13 (2H, d, J=8.9Hz), 7.46 (1H, d, J=8.1Hz), 7.60-7.90 (2H, m), 7.97 (2H, d, J=8.7Hz),8.04-8.14 (4H, m), 8.24-8.27 (1H, m), 8.70-9.00 (2H, m)

MASS (m/z) : 1297.3 ($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{78}N_{10}O_{20}S_2 \cdot 7.5H_2O$:
C 48.56, H 6.53, N 9.76
Found : C 48.56, H 6.31, N 9.63

Example 3

IR (KBr) : 1633.4, 1517.7, 1444.4, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.8Hz), 1.13 (3H, d, J=5.7Hz), 1.20-1.65 (10H, m), 1.65-2.65 (15H, m), 2.70-5.50 (27H, m), 3.21 (3H, s), 4.07 (2H, t, J=6.5Hz), 6.71 (1H, d, J=8Hz), 6.75-6.80 (1H, m), 6.98 (1H, d, J=1.6Hz), 7.13 (2H, d, J=8.9Hz), 7.46 (1H, d, J=8Hz), 7.55-7.85 (2H, m), 7.97 (2H, d, J=8.8Hz), 8.07 (4H, ABq, J=10.8Hz), 8.09-8.13 (1H, m), 8.79 (1H, d, J=7.9Hz), 8.55-9.00 (1H, br s)

MASS (m/z) : 1311.3 ($M-H^+$)

Elemental Analysis Calcd. for $C_{59}H_{80}N_{10}O_{20}S_2 \cdot 10H_2O$:
C 47.45, H 6.75, N 9.38
Found : C 47.68, H 6.27, N 9.21

Example 4

IR (KBr) : 1648.8, 1631.5, 1540.8, 1513.8, 1452.1 cm^{-1}

NMR (DMSO-d_6, δ) : 0.95 (3H, d, J=6.6Hz), 1.07 (3H, d, J=6Hz), 1.1-2.7 (21H, m), 2.7-5.5 (32H, m), 6.68-6.74 (2H, m), 6.9-6.94 (1H, m), 7.13 (2H, d, J=8.9Hz), 7.2-7.5 (1H, m), 7.5-7.8 (2H, m), 7.97

(2H, d, J=8.8Hz), 8.09 (4H, s), 8.29 (1H, d, J=7.3Hz), 8.5-8.9 (2H, m)

MASS (m/z) : 1283.3 ($M-H^+$)

Example 5

IR (KBr) : 1635.3, 1531.2, 1444.4, 1251.6 cm^{-1}

NMR (DMSO-d_6, δ) : 0.96 (3H, d, J=6.7Hz), 1.14 (3H, d, J=6Hz), 1.40-5.30 (41H, m), 3.67-3.70 (2H, m), 4.15-4.23 (2H, m), 6.66 (1H, d, J=8Hz), 6.64-6.72 (1H, m), 6.96 (1H, br s), 7.09 (2H, d, J=8.9Hz), 7.4-7.8 (4H, m), 7.57 (2H, d, J=6.3Hz), 7.74 (2H, d, J=8.8Hz), 8.0-8.3 (7H, m), 8.73 (1H, d, J=7.5Hz)

MASS (m/z) : 1304.3 (M^+)

Elemental Analysis Calcd. for $C_{59}H_{72}N_{10}O_{20}S_2 \cdot 11H_2O$:
C 47.13, H 6.30, N 9.32
Found : C 47.22, H 5.90, N 8.82

Example 6

IR (KBr) : 1635.3, 1531.2, 1508.1, 1444.4, 1251.6 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.7Hz), 1.14 (3H, d, J=5.4Hz), 1.3-5.3 (44H, m), 3.25 (3H, s), 4.05 (2H, t, J=6Hz), 6.70 (1H, d, J=8.2Hz), 6.75-6.79 (1H, m), 6.96 (1H, br s), 7.07 (2H, d, J=8.9Hz), 7.4-7.8 (4H, m), 7.73 (2H, d, J=8.8Hz), 7.87 (2H, d, J=8.5Hz), 8.08-8.16 (7H, m), 8.7-8.8 (1H, m)

MASS (m/z) : 1332.4 (M^+)

Elemental Analysis Calcd. for $C_{61}H_{76}N_{10}O_{20}S_2 \cdot 11H_2O$:
C 47.84, H 6.45, N 9.15
Found : C 48.10, H 6.00, N 8.94

Example 7

IR (KBr) : 1635.3, 1606.4, 1531.2, 1496.5, 1444.4, 1419.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.8Hz), 1.11 (3H, d, J=5.7Hz), 1.05-1.04 (5H, m), 1.50-5.30 (52H, complex m), 6.67 (1H, d, J=5.7Hz), 6.73-6.80 (1H,

m), 7.01 (1H, d, J=1.6Hz), 7.08 (2H, d, J=9Hz), 7.4-7.8 (3H, m), 7.85 (2H, d, J=8.7Hz), 8.07 (4H, ABq, J=9Hz), 8.31 (1H, d, J=6.9Hz), 8.71 (1H, s), 8.91 (1H, d, J=7.4Hz)

MASS (m/z) : 1319.4 ($M-H^+$)

Elemental Analysis Calcd. for $C_{60}H_{80}N_{12}O_{18}S_2 \cdot 9H_2O$:
C 48.57, H 6.66, N 11.33

Found : C 48.77, H 6.54, N 11.25

Example 8

IR (KBr) : 1635.3, 1529.3, 1519.6, 1467.6, 1446.4, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.91 (3H, t, J=7Hz), 0.96 (3H, d, J=8.3Hz), 1.12 (3H, d, J=5.6Hz), 1.2-2.6 (17H, m), 2.6-5.4 (29H, m), 6.71 (1H, d, J=8Hz), 6.77 (1H, br d, J=8Hz), 6.98 (1H, d, J=1.7Hz), 7.14 (2H, d, J=8.9Hz), 7.45 (1H, d, J=8.5Hz), 7.4-7.8 (3H, m), 7.90 (2H, d, J=8.8Hz), 8.05 (4H, s), 8.1-8.3 (1H, s), 8.64 (1H, d, J=6.9Hz), 8.85 (1H, s)

MASS (m/z) : 1278.3 ($M-H^+$)

Elemental Analysis Calcd. for $C_{57}H_{73}N_{11}O_{19}S_2 \cdot 9H_2O$:
C 47.46, H 6.36, N 10.68

Found : C 47.58, H 6.17, N 10.62

Example 9

IR (KBr) : 3361.3, 2937.1, 1635.3, 1523.5, 1461.8, 1251.6 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.8Hz), 1.10 (3H, d, J=5.9Hz), 1.2-5.3 (49H, m), 6.67-6.80 (2H, m), 7.01 (1H, d, J=1.6Hz), 7.15 (2H, d, J=9Hz), 7.4-7.8 (3H, m), 7.88 (2H, d, J=8.8Hz), 7.96 (4H, s), 8.35 (1H, d, J=8.3Hz), 8.7-8.8 (2H, m), 8.86 (1H, s)

API-ES MASS (Negative) : 1290.3 ($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{73}N_{11}O_{19}S_2 \cdot 8H_2O$:
C 48.29 H 6.26, N 10.53

Found : C 48.49 H 6.24, N 10.73

Example 10

IR (KBr) : 1637.3, 1523.5, 1459.9, 1238.1 cm^{-1}

MASS (m/z) : 1358.4 ($M-H^+$)

Example 11

IR (KBr) : 3357.5, 1631.5, 1517.7, 1465.6, 1450.2, 1241.9 cm^{-1}

NMR (DMSO-d_6, δ) : 0.98 (3H, d, J=6.8Hz), 1.10 (3H, d, J=6Hz), 1.18 (6H, d, J=6Hz), 1.5-2.7 (11H, m), 2.8-5.4 (33H, m), 6.71 (1H, d, J=8.2Hz), 6.78 (1H, dd, J=8 and 1.6Hz), 7.01 (1H, d, J=1.6Hz), 7.12 (2H, d, J=9Hz), 7.44 (1H, d, J=8.7Hz), 7.6-7.9 (1H, m), 7.67 (1H, d, J=8Hz), 7.78 (2H, d, J=8.8Hz), 7.96 (4H, s), 8.35 (1H, d, J=7Hz), 7.6-8.8 (1H, br s), 8.75 (1H, d, J=7Hz), 8.81 (1H, s)

API-ES MASS (Negative) : 1305.3($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{74}N_{12}O_{19}S_2 \cdot 8H_2O$:
C 48.05, H 6.24, N 11.55

Found : C 47.99, H 6.25, N 11.58

Example 12

IR (KBr) : 1631.5, 1510.0, 1446.4, 1234.2 cm^{-1}

NMR (DMSO-d_6, δ) : 0.96 (3H, d, J=6.7Hz), 1.09 (3H, d, J=5.8Hz), 1.2-2.65 (15H, m), 2.7-5.3 (41H, m), 3.21 (3H, s), 3.30 (2H, t, J=6.4Hz), 3.85 (2H, t, J=6.5Hz), 6.70 (1H, d, J=8.2Hz), 6.74-6.80 (1H, m), 6.83 (2H, d, J=9Hz), 6.94 (2H, d, J=9Hz), 6.99 (1H, s), 7.01 (2H, d, J=8.8Hz), 7.44 (1H, d, J=8.6Hz), 7.6-7.9 (2H, m), 7.80 (2H, d, J=8.7Hz), 8.1-8.3 (2H, m), 8.37 (1H, d, J=7.7Hz)

MASS (m/z) : 1297.5 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{60}H_{86}N_{10}O_{20}S \cdot 7H_2O$:
C 50.55, H 7.07, N 9.83

Found : C 50.68, H 7.08, N 9.82

Example 13

IR (KBr) : 1648.8, 1631.5, 1540.8, 1511.9, 1454.1, 1238.1 cm^{-1}

NMR (DMSO-d_6, δ) : 0.8-1.3 (18H, m), 1.5-2.5 (24H, m), 2.61 (4H, br s), 2.8-5.4 (27H, m), 6.70 (1H, d, J=8.1Hz), 6.77 (1H, br d, J=10Hz), 6.92 (2H, d, J=9Hz), 7.00 (1H, d, J=1.6Hz), 7.42 (1H, d, J=8.6Hz), 7.5-7.7 (2H, m), 7.76 (2H, d, J=8.6Hz), 8.30 (1H, d, J=7.1Hz), 8.44 (1H, d, J=6.9Hz), 8.46-9.00 (1H, br s)

MASS (m/z) : 1241.3($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{86}N_{10}O_{18}S \cdot 10H_2O$:
C 48.94, H 7.50, N 9.84
Found : C 49.19, H 7.33, N 9.73

Example 14

A solution of Starting compound (150 mg) in N,N-dimethylformamide (1.5 ml) was treated with diisopropylethylamine (166.5 mg) and ethyl formimidate hydrochloride (64.8 mg) and stirred 2 days at room temperature. Additional ethyl formimidate hydrochloride (39 mg) was added and stirring continued a further 3 hours 15 minutes. The reaction mixture was diluted with water and prified by ODS column chromatography, eluting with aqueous acetonitrile. Product-containing fractions were pooled, evaporated, and lyophilized to give Object compound as an amorphous white powder.

IR (KBr) : 1658.5, 1635.3, 1444.4, 1257.4 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.7Hz), 1.10 (3H, d, J=6.1Hz), 1.20-1.60 (8H, m), 1.60-2.50 (15H, m), 3.21 (3H, s), 2.80-5.30 (27H, m), 6.71 (1H, d, J=8.1Hz), 6.78 (1H, d, J=6Hz), 7.00 (1H, br s), 7.14 (2H, d, J=8.9Hz), 7.40-7.84 (4H, m), 7.84 (1H, s), 7.97 (2H, d, J=8.8Hz), 8.08 (4H, ABq, J=8.9Hz), 8.30-8.40 (2H, m), 8.90-9.10 (2H, m)

MASS (m/z) : 1325.4 ($M-H^+$)

Elemental Analysis Calcd. for $C_{58}H_{79}N_{11}O_{19}S_2 \cdot 8H_2O$:

C 48.29, H 6.64, N 10.68

Found : C 48.01, H 6.34, N 10.38

The following compounds [Examples 15 to 17] were obtained according to a similar manner to that of Example 14.

Example 15

IR (KBr) : 1658, 1635, 1628, 1444, 1257 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.7Hz), 1.11 (3H, d, J=6.1Hz), 1.25-1.60 (8H, m), 1.60-2.50 (15H, m), 2.05 (3H, s), 3.21 (3H, s), 2.80-5.30 (27H, m), 6.71 (1H, d, J=8.1Hz), 6.77 (1H, d, J=8Hz), 7.00 (1H, br s), 7.13 (2H, d, J=8.9Hz), 7.30-7.90 (4H, m), 7.97 (2H, d, J=8.7Hz), 8.08 (4H, ABq, J=8.8Hz), 8.50-9.00 (4H, m)

MASS (m/z) : 1362.3 ($M-Na^+$)

Elemental Analysis Calcd. for $C_{59}H_{81}N_{11}O_{19}S_2 \cdot 9H_2O$:

C 48.06, H 6.77, N 10.45

Found : C 48.02, H 6.48, N 10.11

Example 16

IR (KBr) : 1643.1, 1633.4, 1535.1, 1513.8, 1442.5, 1249.6 cm^{-1}

NMR (DMSO-d_6, δ) : 0.97 (3H, d, J=6.8Hz), 1.1-1.16 (3H, m), 1.12 (3H, t, J=7Hz), 1.4-2.6 (12H, m), 2.8-5.2 (34H, m), 6.71 (1H, d, J=8Hz), 6.78 (1H, dd, J=8 and 2Hz), 7.00 (1H, d, J=2Hz), 7.08 (2H, d, J=8.8Hz), 7.45 (1H, d, J=8.9Hz), 7.6-7.8 (2H, m), 7.73 (2H, d, J=8.8Hz), 7.87 (2H, d, J=8.5Hz), 8.0-8.2 (6H, m), 8.28 (1H, d, J=7Hz), 8.91 (1H, d, J=7.6Hz), 8.5-9.05 (1H, br s)

MASS (m/z) : 1331.2 ($M-H^+$)

Elemental Analysis Calcd. for $C_{61}H_{76}N_{10}O_{20}S_2 \cdot 10H_2O$:

C 48.41, H 6.39, N 9.25

Found : C 48.63, H 6.13, N 9.13

Example 17

IR (KBr) : 1631.5, 1537.0, 1510.0, 1448.3, 1234.2 cm^{-1}

NMR (DMSO-d_6+D_2O, δ) : 0.96 (3H, d, J=6.7Hz), 1.05-1.15 (3H, m), 1.2-3.0 (33H, m), 3.15 (3H, s), 3.29 (2H, t, J=6.4Hz), 3.88 (2H, t, J=6.4Hz), 3.6-4.5 (14H, m), 4.7-4.85 (2H, m), 6.73-7.04 (9H, m), 7.75-7.9 (2H, m)

MASS (m/z) : 1311.4 ($M-H^+$)

Elemental Analysis Calcd. for $C_{61}H_{88}N_{10}O_{20}S \cdot 10H_2O$:
C 49.05, H 7.29, N 9.38

Found : C 48.78, H 6.83, N 9.27

Example 18

To a solution of a mixture of the starting compound (18) (5.4 g), 2-oxo-1,3-diacetoxypropane (4.85 g) and acetic acid (0.78 ml) in a mixture of methanol (80 ml) and dimethylformamide (40ml) was added sodium cyanoborohydride (1.71 g) with stirring at ambient temperature, and the mixture was stirred at the same temperature overnight. The reaction mixture was concentrated in vacuo. To the resulting residue was added pH 6.86 standard buffer solution (100 ml) and acetonitrile (20 ml), and the solution was adjusted pH to 8.5 with 1N sodium hydroxide. The solution was subjected to column chromatography on ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (400 ml) eluting in turn with water, 20% acetonitrile in water and 25% acetonitrile in water. The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (18) (4.44 g).

IR (KBr): 1632, 1516, 1452, 1273, 1248 cm^{-1}

NMR (DMSO-d6+D2O, δ): 0.98 (3H, d, J=6.88Hz), 1.11 (3H, d, J=5.64Hz), 1.36 (9H, S), 1.40-2.00 (6H, m), 2.50-2.95 (4H, m), 3.30-3.55 (2H, m), 3.65-4.45 (16H, m), 4.70-4.85 (2H, m), 5.36 (1H, S), 6.71 (1H, d, J=8.05Hz), 6.77 (1H, d, J=8.29Hz), 6.99 (1H, S), 7.30-7.45 (5H, m)

APCI MASS (Positive): (m/z) 1175.4 (M^++Na)

Elemental analysis Calcd. for $C_{50}H_{72}N_8O_{21}S \cdot 5H_2O$
: C 46.80, H 6.52, N 8.73
Found : C 47.06, H 6.44, N 8.54

Example 19

A solution of the starting compound (19) (4.42 g) and 10% palladium on carbon (50% including water) (3.0 g) in a mixture of methanol (90 ml) and water (80 ml) was hydrogenated under an atmospheric pressure of hydrogen with stirring at ambient temperature for 8 hours. To the reaction mixture was added 10% palladium hydroxide on carbon (50% including water) (4.0 g),

and, the mixture was hydrogenated under an atmospheric pressure of hydrogen with stirring at ambient temperature for 16 hours. The catalyst was filtered off and washed with a mixture of methanol and water (1:1 v/v) (50 ml), and the filtrate and washes were combined. To the solution was dropwise added allyloxycarbonyl chloride (1.72 ml) in tetrahydrofuran (4 ml) adjusting pH to 8.5-10.0 with 1N sodium hydroxide with stirring on an ice-bath. The mixture was stirred at the same temperature for 2 hours and adjusted pH to 8.0 with 1N hydrochloric acid. The solution was subjected to column chromatography on ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (400 ml) eluting with 10% acetonitrile in water and then with 20% acetonitrile in water. The first fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the major object compound (50) (0.47 g). The second fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the minor object compound (50) (2.91 g).

major object compound (50)

IR (KBr): 1761, 1672, 1635, 1512, 1450 cm^{-1}

NMR (DMSO-d6+D2O, δ): 0.96 (3H, d, J=6.79Hz), 1.00-1.15 (3H, m), 1.35 (9H, S), 1.45-2.50 (9H, m), 2.80-3.40 (6H, m), 3.70-4.60 (16H, m), 4.65-4.90 (4H, m), 5.10-5.45 (4H, m), 5.80-6.10 (2H, m), 6.71 (1H, d J=8.23Hz), 6.77 (1H, d. J=9.01Hz), 6.98 (1H, S)

ESI MASS (Positive): (m/z) 1277.2 (M^++Na)

minor object compound (50)

NMR (DMSO-d6+D2O, δ): 0.96 (3H, d, J=6.57Hz), 1.06 (3H, d J=4.94Hz), 1.36 (9H, S), 1.45-2.45 (8H, m), 2.75-3.70 (9H, m), 3.75-4.60 (12H, m), 4.69 (2H, d J=5.19Hz), 4.70-4.90 (2H, m), 5.05-5.50 (3H, m), 5.80-6.10 (1H, m), 6.91 (1H, d, J=8.29Hz), 7.10 (1H,

d, J=8.31Hz), 7.43 (1H, S)
ESI MASS (Positive): (m/z) 1193.3 (M^++Na)

Example 20

A suspension of the object compound (20) (1.73 g) in dichloromethane (40 ml) was stirred with cooling at 5 ℃ and treated with triethylsilane (1.1 ml), followed by trifluoroacetic acid (3.19 ml) dropwise over 30 minutes. After warming to room temperature, the clear solution was stirred for 2 hours, and then poured into a mixture of saturated aqueous sodium hydrogen carbonate (100 ml) and pH 6.86 standard buffer (100 ml). Organic solvent was removed by evaporation, and the remaining aqueous solution purified by ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (200 ml) column chromatography, eluting with aqueous acetonitrile (10-20%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (20) (1.10 g).

IR (KBr): 1761, 1668, 1647, 1539, 1512, 1437 cm^{-1}
NMR (DMSO-d6+D2O, δ): 0.95 (3H, d, J=6.77Hz), 1.18 (3H, d, J=4.94Hz), 1.40-2.40 (7H, m), 2.70-3.40 (4H, m), 3.60-4.60 (17H, m), 4.69 (2H, d, J=5.37Hz), 4.70-4.90 (2H, m), 5.10-5.50 (4H, m), 5.80-6.20 (2H, m), 6.89 (1H, d), 7.08 (1H, d, J=8.21Hz)
ESI MASS (Positive): (m/z) 1155.4 (M^++Na)
Elemental analysis Calcd. for $C_{46}H_{68}N_8O_{23}S \cdot 4H_2O$
: C 45.84, H 6.36, N 9.30
Found : C 45.85, H 6.33, N 9.16

Example 21

A solution of the starting compound (21) (0.43 g) in dimethylformamide (4 ml) was treated with 4-[2-[4-[4-(5-methoxypentyloxy)piperidin-1-yl]phenyl]imidazo[2,1-b][1,3,4]thiadiazol-6-yl]benzoic acid benzotriazol-1-yl ester (194 mg) and diisopropylethylamine (78.4 μl) and stirred

for 5 hours at room temperature. Ethylacetate (50 ml) was added, and the resulting precipitate collected, washed with isopropylether, and dried to give the object compound (21) (610.6 mg) as a crude powder, that was used directly in the next reaction without purification.

Example 22

To a solution of the starting compound (22) (610.6 mg) in a mixture of methanol (10 ml) and tetrahydrofuran (25 ml) were successively added triphenylphosphine (32 mg), tetrakis(triphenylphosphine)palladium(0) (35 mg) and morpholine (106 μl) with stirring, and the mixture was stirred at ambient temperature for 3.5 hours. Ethyl acetate (100 ml) was added, and the resulting precipitate collected, washed with isopropylether, and dried to give a crude pale yellow powder (535 mg). The crude powder was dissolved sodium hydroxide aqueous solution and subjected to column chromatography on ODS(YMC-gel ODS-AM-S-50 (Trademark: prepared by YMC Co. Ltd.)) (37% acetonitrile aqueous solution). The fractions containing the object compound were combined, and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (22)(293.7 mg).

IR(KBr): 3355.5, 1633.4, 1608.3, 1529.3, 1517.7, 1463.7, 1444.4, 1267.0, 1230.4 cm^{-1}

NMR(DMSO-d6, δ): 0.98(d, 3H, J=6.7Hz), 1.10(d, 3H, J=5.6Hz), 1.2-5.6 (m, 65H), 6.71(d, 1H, J=8.1Hz), 6.78(d, 1H, J=9.7Hz), 7.00(s, 1H), 7.09(d, 2H, J=9.1Hz), 7.75(d, 2H, J=8.7Hz), 7.95(s, 4H), 7.3-8.7(m, 7H), 8.79(s, 1H)

MASS: (m/z) 1465.5 $(M-H)^-$

Elemental analysis Calcd. for $C_{66}H_{90}N_{12}O_{22}S_2 \cdot 7H_2O$:
C 49.74, H 6.58, N 10.55
Found : C 49.72, H 6.43, N 10.40

Example 23

A solution of the starting compound (10 g) in a mixture

of methanol(500 ml) and water (100 ml) was treated with cobalt (II) chloride hexahydrate (9.43 g) and then stirred to give a pink solution. Sodium borohydride (7.5 g) was then added portionwise and stirred for 1 hour at ambient temperature. The reaction mixture was filtered through a bed of celite, washing with a mixture of methanol (100 ml) and water (20 ml). The ice-cooled filtrate was then treated dropwise with a solution of allyloxycarbonyl chloride (1.46 ml) in tetrahydrofuran (10 ml), keeping pH 8.0-9.5 with 1N sodium hydroxide and then stirred for 1 hour at the same temperature. The reaction mixture was evaporated in vacuo (about 200 ml) and added 1N sodium hydroxide (60 ml), and then the mixture was stayed in the refrigerator overnight. To the solution was added water (200 ml), and the mixture was purified by ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark : prepared by Daiso Co. Ltd.)) (200 ml) column chromatography, eluting with aqueous acetonitrile (5-20%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (23) (8.58 g).

IR (KBr): 1670, 1633, 1516, 1443, 1269 cm^{-1}

NMR (DMSO-d6+D2O, δ) : 0.97 (3H, d, J=6.75Hz), 1.08 (3H, d, J=5.52Hz), 1.35 (9H, S), 1.40-2.00 (6H, m), 2.10-2.50 (3H, m), 2.80-3.40 (4H, m), 3.65-4.50 (14H, m), 4.65-4.85 (2H, m), 5.05-5.35 (2H, m), 5.70-6.00 (1H, m), 6.72 (1H, d, J=8.12Hz), 6.78 (1H, d, J=10.1Hz)

ESI MASS (Positive): (m/z) 1119.3 (M^++Na)

Elemental analysis Calcd. for $C_{45}H_{67}N_8O_{21}SNa \cdot 5H_2O$:
C 44.52, H 6.37, N 9.44
Found : C 44.59, H 6.43, N 9.47

Example 24

A suspension of the starting compound (24) (8.5 g) in dichloromethane (180 ml) was stirred with cooling at 5 ℃ and treated with triethylsilane (6.2 ml), followed by trifluoroacetic acid (17.9 ml) dropwise over 30 minutes.

After warming to room temperature, the clear solution was stirred for 2 hours, then poured into a mixture of saturated aqueous sodium hydrogen carbonate (200 ml) and pH 6.86 standard buffer (200 ml). Organic solvent was removed by evaporation, and the remaining aqueous solution purified by ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (200 ml) column chromatography, eluting with aqueous acetonitrile (5-20%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (24) (5.53 g).

NMR (DMSO-d6+D2O, δ): 0.97 (3H, d, J=6.64Hz), 1.15 (3H, d, J=5.52Hz), 1.30-1.70 (3H, m), 1.80-2.50 (6H, m), 2.70-4.00 (14H, m), 4.20-4.60 (8H, m), 4.70-4.90 (2H, m), 5.10-5.40 (2H, m), 5.70-6.10 (1H, m), 6.70-6.90 (2H, m), 7.06 (1H, S)

ESI MASS (Positive): (m/z) 997.3 (M^++Na)

Example 25

A solution of the starting compound (25) (0.5 g) in dimethylformamide (10 ml) was treated with 4-[5-[4-[4-(cis-4-methylcyclohexyl)piperazinyl]phenyl]-1,3,4-thiadiazol-2-yl]benzoic acid benzotriazol-1-yl ester (0.3 g) and diisopropylethylamine (0.13 ml) and stirred for 20 hours at room temperature. Ethylacetate (100 ml) was added and the resulting precipitate collected, washed with ethylacetate, and dried to give the object compound (25) (0.5 g).

NMR (DMSO-d6, δ): 0.90 (3H, d, J=6.8Hz), 0.97 (3H, d, J=6.6Hz), 1.13 (3H, d, J=5.0Hz), 1.43-6.10 (78H, m), 6.69-8.72 (18H, m)

ESI MASS (Negative): (m/z) 1418.4 (M^+)

Example 26

To a suspension of the starting compound (26) (0.38 g) in a mixture of methanol (7.6 ml) and tetrahydrofuran (1.9 ml) were successively added triphenylphosphine (0.04 g),

tetrakis(triphenylphosphine)palladium(O) (0.088 g) and morpholine (0.14 ml) with stirring and the mixture was stirred at ambient temperature for 15 hours. To the reaction mixture was added ethylacetate (100 ml). The resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of water and 1N sodium hydroxide and the solution was subjected to column chromatography on ODS(Daiso-gel, SP-120-40/60-ODS-B(Trade mark: prepared by Daiso Co. Ltd.)) (100 ml) eluting with 40% acetonitrile in water. The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (26) (0.25 g).

NMR (DMSO-d6, δ): 0.90 (3H, d, J=6.7Hz), 0.98 (3H, d, J=6.7Hz), 1.11 (3H, d, J=5.7Hz), 1.42-5.23 (56H, m), 6.69-8.92 (17H, m)

ESI MASS (Negative): (m/z) 1334.4 (M^+)

Elemental analysis Calcd. for $C_{61}H_{82}N_{12}O_{18}S_2 \cdot 8H_2O$:

C 49.52, H 6.68, N 11.36

Found : C 49.25, H 6.41, N 11.20

Example 27

The suspension of a mixture of the starting compound (27) (100 mg), 1,3-dihydroxyacetate (13.5 mg) and acetic acid (00.13 ml) in a mixture of methanol (1.5 ml) and dimethylformamide (0.7 ml) was added sodium cyanoborohydride (9.4 mg) with stirring at ambient temperature, and the mixture was stirred at the same temperature overnight. To the reaction mixture was added ethylacetate (20 ml). The resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of water and 1N sodium hydroxide and the solution was subjected to column chromatography on ODS(Daiso-gel, SP-120-40/60-ODS-B(Trade mark: prepared by Daiso Co. Ltd.)) (50 ml) eluting with 40% acetonitrile in water. The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was

lyophilized to give the object compound (27) (55 mg).

NMR (DMSO-d6, δ): 0.90 (3H, d, J=6.8Hz), 0.98 (3H, d, J=6.7Hz), 1.11 (3H, d, J=5.5Hz), 1.43-5.24 (62H, m), 6.69-8.85 (17H, m)

ESI MASS (Negative): (m/z) 1408.3 (M^+)

Example 28

To a solution of a mixture of the starting compound (28) (7.5 g), 1,3-dihydroxyacetone (1.19 g) and acetic acid (1.14 ml) in a mixture of methanol (120 ml) and dimethylformamide (55 ml) was added sodium cyanoborohydride (835 mg) with stirring at ambient temperature, and the mixture was stirred at the same temperature overnight. To a reaction mixture was poured into ethyl acetate (700 ml). The resulting precipitates were collected by filtration, washed with ethyl acetate (100 ml) and dried in vacuo. The precipitates were dissolved in a mixture of 30% aqueous acetonitrile (800 ml) and 1N sodium hydroxide (5 ml). The solution was subjected to column chromatography on ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (440 ml) eluting in turn with water and aqueous acetonitrile (30%-60%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (28) (5.22 g).

IR (KBr): 1632, 1535, 1518, 1443, 1269, 1082, 1047 cm-1

NMR (DMSO-d6+D2O, δ): 0.82 (3H, d, J=6.83Hz), 0.97 (3H, d, J=6.81Hz), 1.02 (3H, d, J=6.18Hz), 1.24 (26H, S), 1.35-2.45 (14H,m), 2.75-3.40 (5H, m), 3.60-4.50 (15H, m), 4.70-4.90 (2H, m), 6.65-6.80 (2H, m), 7.01 (1H, S)

ESI MASS (Positive): (m/z) 1088.4 (M^++Na)

Example 29

To a solution of the starting compound (29) (4.0 g) in dimethylformamide (40 ml) were successively added diisopropylethylamine (1.45 ml) and 9-fluorenylmethyl

chloroformate (1.03 g), and the mixture was stirred at ambient temperature for 2 hours. The reaction mixture was poured into water (200 ml). The solution was purified by ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (200 ml) column chromatography, eluting in turn with a mixture of saturated aqueous sodium chloride (400 ml), saturated aqueous sodium hydrogen carbonate (50 ml) and water (400 ml), and aqueous acetonitrile (30-60%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give the object compound (29) (2.82 g).

IR (KBr): 1666, 1632, 1518, 1446, 1273, 1246, 1082, 1047 cm-1

NMR (DMSO-d6+D2O, δ): 0.80-1.10 (9H, m), 1.23 (26H, S), 1.35-2.45 (12H, m), 2.60-3.40 (6H, m), 3.60-4.55 (18H, m), 4.65-4.90 (2H,m), 6.65-6.85(2H, m), 6.97 (1H, S), 7.30-7.50 (4H, m), 7.60-7.95 (4H, m)

ESI MASS (Negative): (m/z) 1423.7 (M^+-Na)

Elemental analysis Calcd. for $C_{69}H_{99}N_8O_{22}SNa \cdot 6H_2O$: C 53.27, H 7.19, N 7.20

Found: C 53.45, H 7.21, N 7.10

Example 30

To a solution of the object compound (30) (1.21 g) in dimethylformamide (15 ml) were successively added diisopropylethylamine (0.26 ml) and di-tert-butyl dicarbonate (285 mg), and the mixture was stirred at ambient temperature overnight. The reaction mixture was poured into a mixture of pH6.86 standard buffer solution (150 ml), saturated aqueous sodium chloride (50 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The mixture was purified by ODS (Daiso-gel, SP-120-40/60-ODS-B (Trade mark: prepared by Daiso Co. Ltd.)) (200 ml) column chromatography, eluting with aqueous acetonitrile (30-50%). The fractions containing the object compound were collected and evaporated under reduced pressure to remove acetonitrile. The residue was lyophilized to give

the object compound (30) (1.19 g).

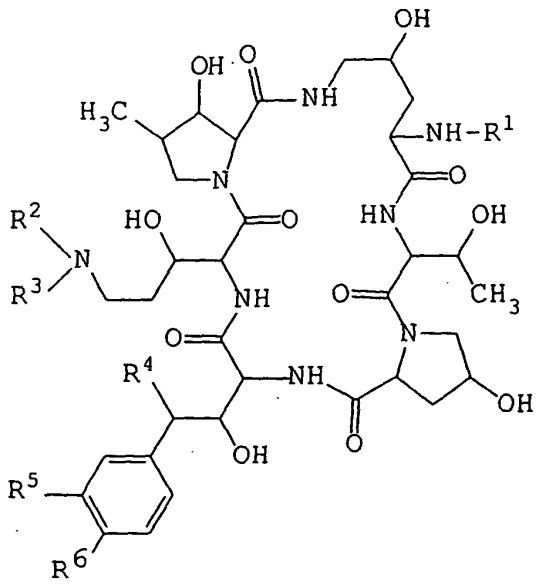
IR (KBr) : 1662, 1632, 1535, 1518, 1444, 1367, 1272, 1250 cm-1

NMR (DMSO-d6+D2O, δ): 0.85 (3H, d, J=6.76Hz), 0.96 (3H, d, J=6.77Hz), 1.04 (3H, d, J=5.50Hz), 1.23 (26H, S), 1.37 (9H,S), 1.40-1.50 (2H, m), 1.55-2.50 (10H, m), 2.80-3.40 (6H, m), 3.50-4.45 (14H,m), 6.65-6.80(2H, m), 6.96 (1H, S)

ESI MASS (Negative): (m/z) 1301.6 (M^+-Na)

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A polypeptide compound of the following general formula (I) :



(I)

wherein

R^1 is hydrogen or acyl group,

R^2 and R^3 are independently hydrogen, lower alkyl which may have one or more suitable substituent(s) or acyl group,

R^4 is hydrogen or hydroxy,

R^5 is hydrogen, hydroxy or hydroxysulfonyloxy and,

R^6 is hydroxy or acyloxy

or a salt thereof.

2. A compound of claim 1, wherein

R^1 is hydrogen or acyl group,

R^2 is hydrogen,

R^3 is lower alkyl which has one or more hydroxy,

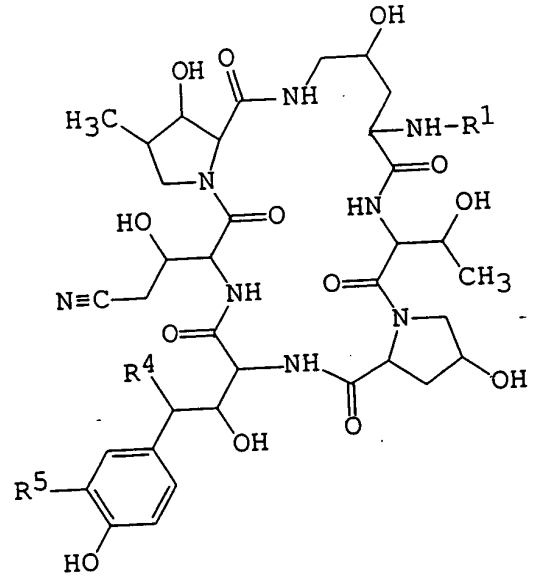
R^4 is hydrogen or hydroxy,

R^5 is hydroxysulfonyloxy and,

R^6 is hydroxy.

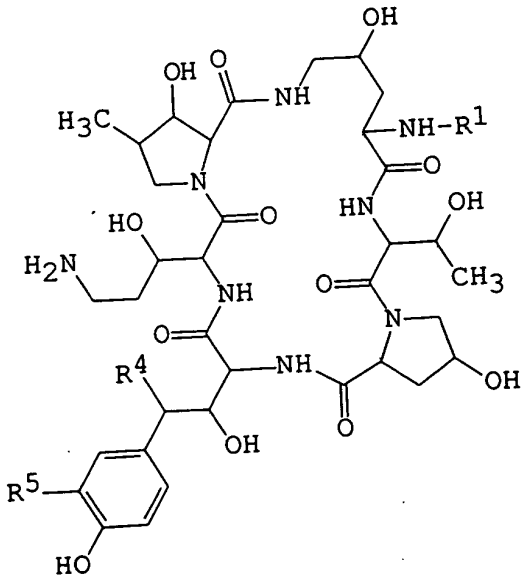
3. A process for preparing a polypeptide compound (I) of claim 1, or a salt thereof,
which comprises,

i) reducing a compound (II) of the formula :



(II)

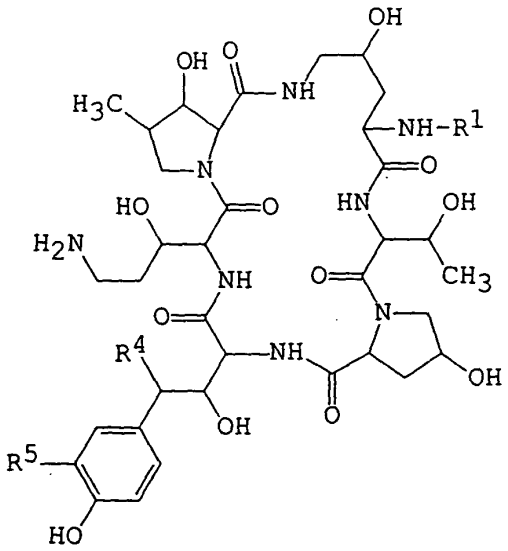
wherein R^1, R^4 and R^5 are as defined in claim 1,
or a salt thereof, to give a compound (Ia) of the formula :



(Ia)

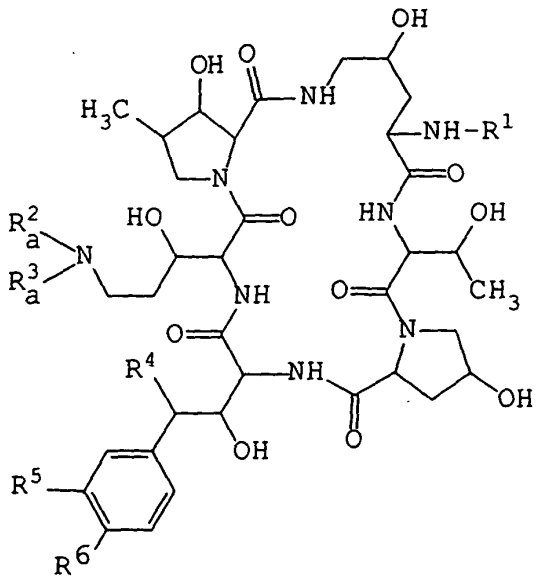
wherein R^1, R^4 and R^5 are as defined in claim 1, or a salt thereof, or

ii) subjecting a compound (Ia) of the formula :



(Ia)

wherein R^1, R^4 and R^5 are as defined in claim 1, or a salt thereof, to protective reaction of amino, to give a compound (Ib) of the formula :



(Ib)

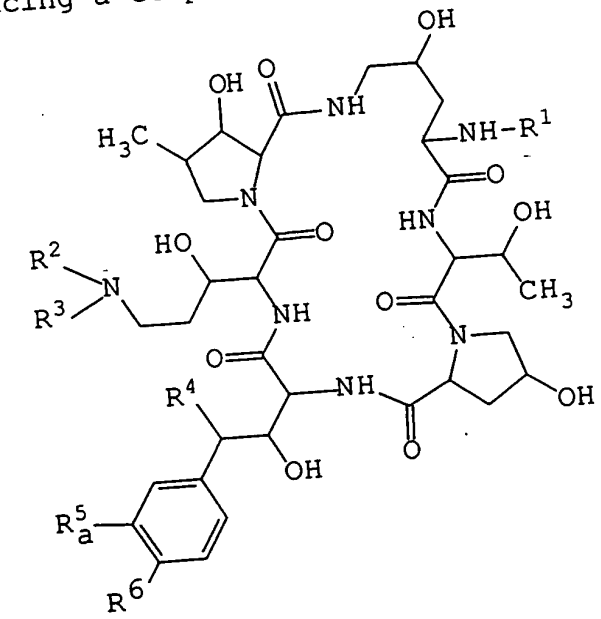
wherein R^1, R^4, R^5 and R^6 are defined in claim 1,

R^2_a is hydrogen, lower alkyl which may have one or more suitable substituent(s) or, acyl group, and

R^3_a is lower alkyl which may have one or more suitable substituent(s), or acyl group,

or a salt thereof, or

iii) reducing a compound (Ic) of the formula :

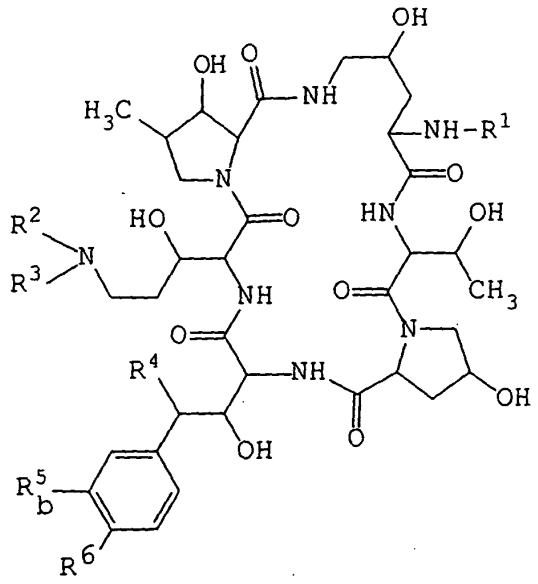


(Ic)

wherein R^1, R^2, R^3, R^4 and R^6 are defined in claim 1, and

R^5_a is hydroxysulfonyloxy,

or a its reactive derivative at the sulfonic acid group, or a salt thereof, to hydrolysis reaction of the sulfonic acid group, to give a compound (Id) of the formula :



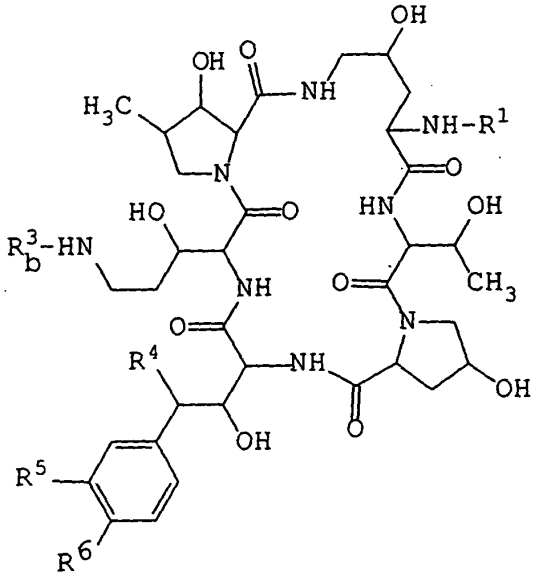
(Id)

wherein R^1, R^2, R^3, R^4 and R^5 are defined in claim 1, and

R^5_b is hydroxy,

or a salt thereof, or

iv) subjecting a compound (Ie) of the formula :



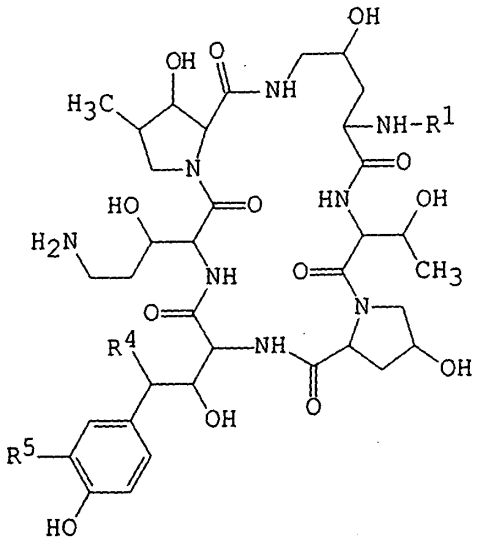
(Ie)

wherein R^1, R^4, R^5 and R^6 are defined in claim 1, and

R^3_b is amino protective group,

or a salt thereof, to elimination reaction of amino protective group, to give a compound (Ia) of the

formula :



(Ia)

wherein R^1, R^4 and R^5 are defined in claim 1, or a salt thereof.

4. A pharmaceutical composition which comprises, as an active ingredient, a compound of Claim 1 or a pharmaceutically acceptable salt thereof in admixture with pharmaceutically acceptable carrier or excipients.

5. Use of a compound of Claim 1 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament.

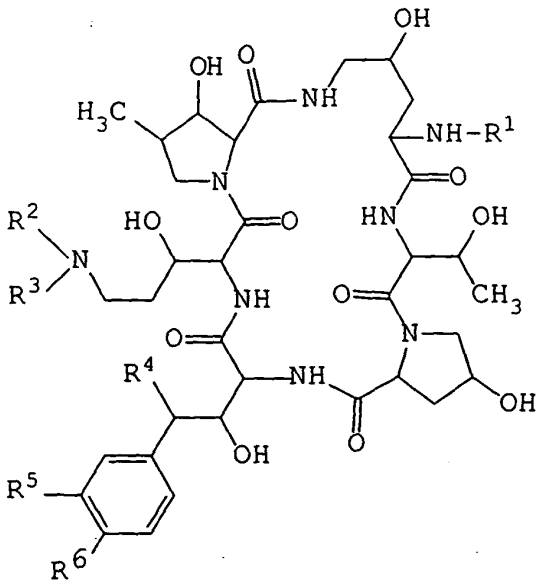
6. A compound of Claim 1 or a pharmaceutically acceptable salt thereof for use as a medicament.

7. A method for the prophylactic and/or therapeutic treatment of infectious diseases caused by pathogenic microorganisms, which comprises administering a compound of claim 1 or a pharmaceutically acceptable salt thereof to a human being or an animal.

Dated this 21st day of February, 2000
Fujisawa Pharmaceutical Co., Ltd.
By DAVIES COLLISON CAVE
Patent Attorneys for the Applicant

A B S T R A C T

This invention relates to new polypeptide compound represented by the following general formula (I) :



(I)

wherein
R^1, R^2, R^3, R^4, R^5 and R^6 are as defined in the description or a salt thereof which has antimicrobial activities (especially, antifungal activities), inhibitory activity on b-1,3-glucan synthase, to process for preparation thereof, to a pharmaceutical composition comprising the same, and to a method for prophylactic and/or therapeutic treatment of infectious diseases including Pneumocystis carinii infection (e.g. Pneumocystis carinii pneumonia) in a human being or an animal.